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Full Length Research Paper

In-vitro plantlet propagation and microtuberization of meristem culture in some of wild and commercial potato cultivars as affected by NaCl

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The effects of *in vitro* salinity (NaCl) on plantlet growth, microtuberization and genetic diversity was investigated under saline and non-saline conditions using twelve cultivars (clones) of potato named Ranger-russet, AGB-69-1, MEX-32, Maine-28, Loman, Araucana INTA, Surena INTA, American-INTA, Aracy, FLS-5, Agria and Marphona, which were planted in the greenhouse. Young shoots were cut and transferred to tissue culture laboratory of agricultural biotechnology research institute. Apical and axillary meristems were cultivated on MS medium and samples were subcultured every four weeks on the same medium for plantlet production. Then plantlets were propagated through single nodal culture. To study the effects of salinity (NaCl) on the growth of single nodal explants and on microtuberization, cultivation on MS media with different concentrations of NaCl (0, 25, 50, 75 and 100 mol l^{-1}) was carried out. Growth of single nodal explants on the saline media indicated that all the characters of the cultivars studied, the salinity levels and the studied interaction effects had significant difference at $P < 0.01$. These characters were also shown to have different responses to salinity levels. Microtuberization responses on different saline media was also different and indicated that all the studied characters, except the number of microtubers and the mean of minimum microtuber diameter, had significant difference at $P < 0.01$ for cultivars, salinity levels and interaction effects. Genetic diversity on the basis of microtuberization using analysis of cluster among potato cultivars and phenotypic correlations among characters under saline and non-saline conditions were also studied.

Key words: Potato, tissue culture, microtuberization, salinity tolerance, genetic diversity.

INTRODUCTION

The salty ground water as well as the irrigating water (containing about 30 g/l of NaCl) tends to limit agricultural activities. However, the use of saline water for crop production is often unavoidable (Heur and Nadler, 1998). Generally, the salinity problem becomes more severe with more intense cultivation (Christiansen and Lewis, 1982). Salinity stress induces several alterations on plant growth, cell division and enzymatic activities, among others. Salinity tolerance of plants primarily depends on the genotype that determines alterations on processes such as uptake and transport of salts by roots, together with metabolic and physiological events occurring at cellular level (Winicov, 1993). A large volume of data has been published on the subject of plant responses to salinity. Salt tolerance is actually a complex phenomenon (Martinez et al., 1996). For the improvement of plants, various techniques have been developed. Novel breeding methods such as somaclonal variation, interspecific hybridization, somatic hybridization and gene transformation provided potential for improving important commercial characters. Today, these unconventional breeding methods combined with conventional methods are used as powerful tools in plant breeding programs by an enormous number of researchers (Turhan, 2005).

Plant tissue culture techniques involve the growing and multiplication of totipotent cells, tissues and organs of plants on defined solid or liquid media comprising nutrients under an aseptic and controlled environment

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(George, 1978). Plant tissue culture has an important role in the production of agricultural or ornamental plants and in the manipulation of plants for improved agronomic performance (Farhachllah et al., 2002).

Potatoes are classified as moderately salt-sensitive and are cultivated in arid and semi-arid regions in many countries, where shortage of water or its poor quality are major factors limiting plant growth and yield (Ahmed and Abdullah, 1982). Although salinity affects a range of developmental responses in potato (Heur and Nadler, 1998), there is still little information on the effects of salt stress on the *in vitro* tuberization. Shoot culture was considered a reliable approach to evaluate *in vitro* saline stress in potatoes (Sasikala and Devi Pasad, 1994; Martinez et al., 1996). Taking into account the various microtuber induction protocols proposed in the literature for several potato species, it may be possible that microtuberization could provide an alternative method of screening potato germplasm for its capacity to tuberize under stress conditions. Indeed, *in vitro* tuberization was considered a potential screening method for heat stress tolerance assessment on nodal explants of potato cultivars (Nowak and Colborne, 1989).

Genetic diversity is the basis of all selections. Data on the relative genetic diversity within and among plant populations may have a major significance in preserving genetic diversity of crops. Improving and maintaining crop germplasm is considered necessary for sustainable agriculture. Much effort is still focused on phenotyping the available genetic diversity of simple traits. Wide range of abiotic stress tolerance in solanum species is interesting, not only for potato breeding for abiotic stresses but also for providing better material to study the abiotic stress tolerance mechanisms (Sasikala and Prasad, 1994; Martinez et al., 1996). The wild cultivated species of potato (*Solanum tuberosum L.*) possess very little abiotic stress tolerance (Martinez et al., 1996), therefore salinity affects a range of developmental responses in potato (Heur and Nadler, 1998).

In this study, genetic diversity and the effect of salinity on the growth and microtuberization of *in vitro* induced nodal explants and plantlets of commercial cultivars and wild cultivars of potato treated with various concentrations of NaCl were studied.

MATERIALS AND METHODS

Twelve cultivars (clones) of potato named Ranger-russet, AGB-69-1, MEX-32, Maine-28, Loman, Araucana INTA, Surena INTA, American-INTA, Aracy, FLS-5, Agria and Marphona (Ranger-russet, Agria and Marphona are commercial cultivars while the rest are wild cultivars) provided by Iranian Research Institute for Agriculture were planted in the greenhouse of the Department of Agronomy and Plant Breeding, University of Tehran, Iran. Young shoots were cut and transferred to Tissue Culture Laboratory of Agricultural Biotechnology Research Institute. Apical and axillary meristems were cultivated on liquid Murashige and Skoog (MS) (1962) medium containing 2.5 mgl^{-1} GA_3 and samples were subcultured every four weeks on the same medium for plantlet production. Then plantlets were propagated using single nodal culture.

For propagating, single nodal explants were cultured on MS medium supplemented with 2.0 mgl^{-1} calcium pantothenate, 2.5 mgl^{-1} GA_3, 30 gl^{-1} sucrose, 7.0 gl^{-1} agar and at pH 5.8 ± 0.1. Cultures were maintained under 16/8 h light/dark photoperiod with 36 $\mu molm^{-2}s^{-1}$ light and the temperature of the growth chamber was kept at 26 ± 2°C.

To culture single node explants (15 - 20 mm length), they were transferred to jars (250 ml capacity) containing 40 ml of the above-mentioned medium supplemented with 0, 25, 50, 75 or 100 $mmoll^{-1}$ NaCl, plus 2.5 mgl^{-1} GA_3, 30 gl^{-1} sucrose at pH 5.8 ± 0.1. Cultures were maintained under 16/8 h light/dark photoperiod with 36 $\mu molm^{-2}s^{-1}$ light. Temperature of the growth chamber was kept at 26 ± 2°C for four weeks. Four nodes were placed in each jar and sealed with polypropylene film. For each cultivar, three vessels (replications) were used for each salinity level. After four weeks, several characters such as shoot length, root length, number of nodes, mean of nodal length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, leaf area, number of leaves and number of branches were measured.

For microtuberization, plantlets were transferred to jars (250 ml capacity) containing 40 ml of the above-mentioned medium supplemented with 0, 25, 50, 75 or 100 mmol l^{-1} NaCl, plus 5 mgl^{-1} BAP, 80 gl^{-1} sucrose and at pH 5.8 ± 0.1. Cultures were grown in complete darkness at 21 ± 1°C in a diurnal growth incubator for two months. Four plantlets were placed in each jar and sealed with polypropylene film. For each cultivar three, vessels (replications) were used for each salinity level.

To study the effects of salinity on the microtuberization eight weeks after cultivation, characters such as the number of microtubers, microtuber fresh weight, microtuber dry weight, the mean of microtuber diameter, the mean number of eyes, the mean of minimum microtuber diameter, the mean of maximum microtuber diameter, the mean number of eyes on the small microtubers and the mean number of eyes on the large microtubers were measured.

Besides, genetic diversity on the basis of microtuberization using analysis of cluster among potato cultivars and also phenotypic correlations among characters under saline and non-saline conditions were studied (that SPSS program is used for data analysis).

RESULTS AND DISCUSSION

Growth of single nodal explants on saline media indicated that all the studied characters had significant difference at $P < 0.01$ for cultivars, salinity levels and interaction effects. These characters showed different responses on different salinity levels (Table 1). Results indicated that control plantlets (grown without NaCl) exhibited large increases in all measured characters over time in all tested cultivars. All measured characters were negatively affected by NaCl. The detrimental effect of salt on the plantlet growth was found to be directly related to its concentration and exposure time.

At the concentration of 100 mmol l^{-1} of NaCl, growth of potato plantlets was severely inhibited. The range of the mortality of the plantlets was positively correlated to the concentration of NaCl. By increasing salinity, rooting was decreased in all cultivars and it was severely inhibited at 100 mmol l^{-1} concentration. By increasing salinity, shoot dry weight was also increased in all cultivars. This observation could be due to ion accumulation. Roots were reported to be among the first organs affected by

Table 1. Comparison of the mean of salinity levels for measured growth characters using Duncan's test at P < 0.01.

Salinity levels	Number of nodes	Mean of nodal length	Shoot length	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	Number of branches	Leaf area	Root length	Number of leaves
0	10.25a	1.003a	9.533a	79.79a	229a	13.59a	20.74abc	10.5a	97.06bc	19.5a	19.89a
25	9.778a	0.7064b	7.039b	15.39b	190.9ab	3.044b	15.28bc	1.611b	70.15c	7.256b	11.58b
50	8.444b	0.5509c	5.017c	12.46bc	156.8abc	2.675b	23.06ab	1.583b	112.8b	6.811bc	10.83b
75	7.778b	0.4099d	3.383d	8.172bc	117.2bc	2.6b	28.28a	0.8333b	172.9a	4.481c	9.139b
100	4.944c	0.2336e	1.764e	1.889c	91.98c	0.4278c	13.51c	0.4167b	30.83d	1.944d	5.694c

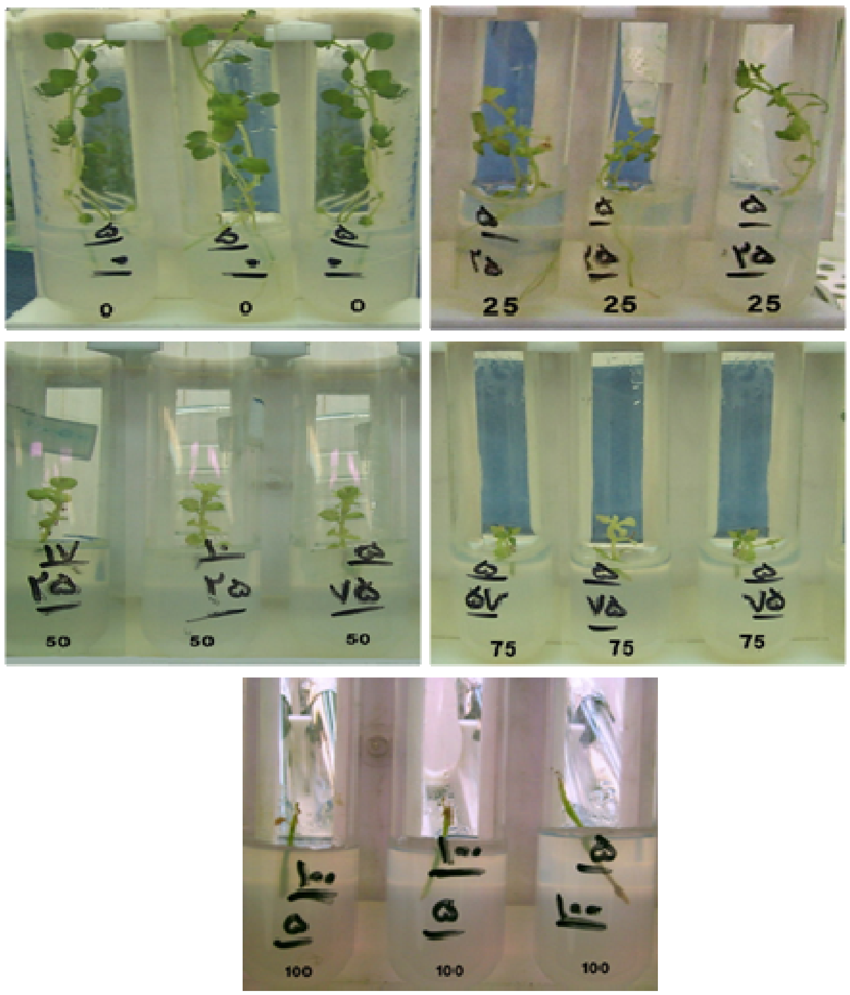

Figure 1. Responses of Loman cultivar under saline conditions (0, 25, 50, 75 and 100 m moll^{-1} NaCl).

salt stress and are the most sensitive. Naik and Widholm (1998) reported severe and specific effects of NaCl on root growth of *in vitro* cultures of potato.

The shoot culture approach, which is based on intact tissue and is, therefore, less prone to result in somaclonal variation, appears to offer a better system for testing and selecting for salt tolerance. On the other hand, potato shoots are easy to propagate *in vitro*, thus, the obtained salt-tolerant plant material can readily be used as established field crops.

The methodology employed in this study allows a comparison between the growth and salt tolerance of different cultivars of potato plantlets. Growth of wild cultivars of potato plantlets in this study were less affected by saline conditions than that of the other genotypes or cultivars (Figure 1).

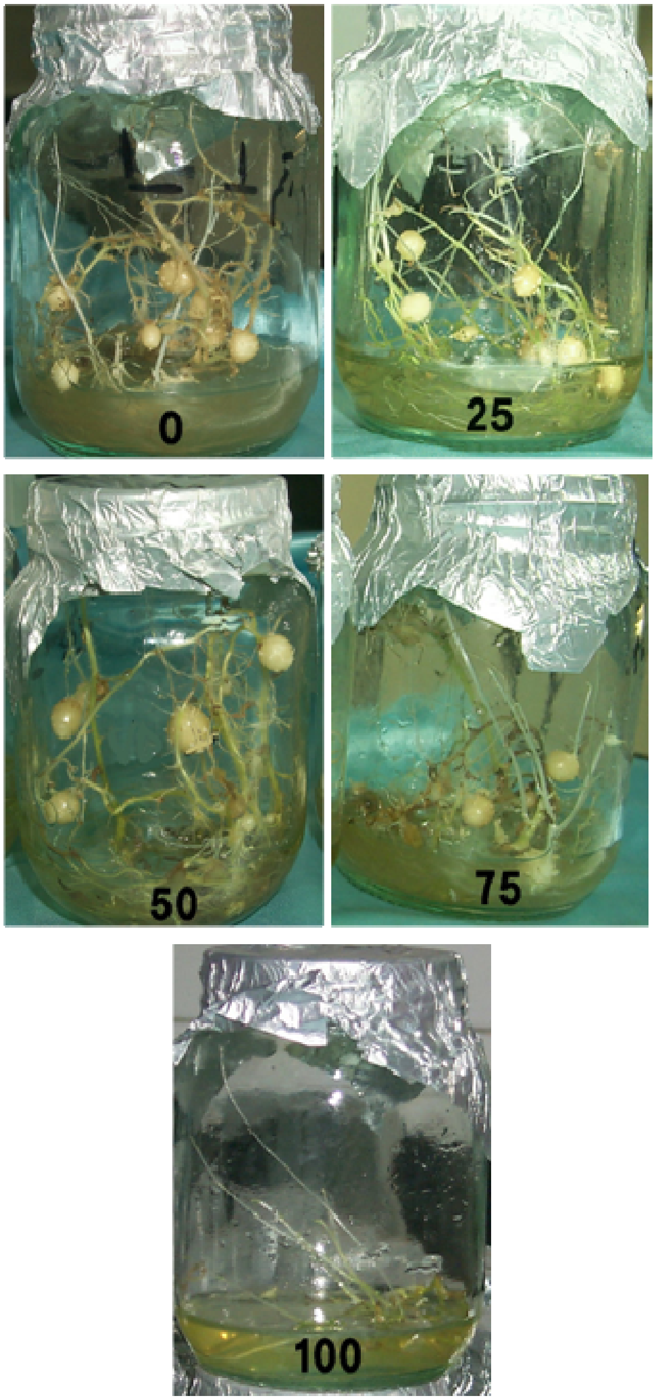

Figure 2. Microtuberization of Surena cultivar under saline conditions (0, 25, 50, 75 and 100 mmoll^{-1} NaCl).

The microtuberization responses after two months on MS-based medium supplemented with different levels of NaCl were different and indicated that all the studied characters, except the number of microtubers and the mean of minimum microtuber diameter, for cultivars, salinity levels and interaction effects had significant difference at $P< 0.01$. All the characters of microtuberization showed higher increase in the absence of salinity.

Additionally, for certain potato cultivars, a radial swelling was observed on the terminal portion of the stolons grown under 100 mmol l^{-1} NaCl. This event is considered as an initial step in microtuberization process (Ulloa et al., 1997). Martinez et al. (1996) evaluated *in vitro* growth characteristics of the potato species and reported contrasting responses to saline stress with a different behavior for each species.

The detrimental effects of salinity on microtuberization of potato cultivars were probably a consequence of the reduction in osmotic potential due to increasing salt levels in cells of both stolon and microtuber tissues. Salinity possibly causes a reduction of water content and nutrient uptake in microtubers, leading to an increase in dry weight of potato (Silva et al., 2001).

The highest salinity level (100 mmol l^{-1} NaCl) completely inhibited tuber development for all species, but not stolon growth. As stated by Nowak and Colborne (1989), *in vitro* tuberization was considered a potential screening method for salinity stress tolerance using nodal explants derived from wild cultivars of potato. Salinity stress significantly reduced microtuberization but the degree of response varied among the cultivars. The results of this study suggest a marked genetic difference among the tested genotypes. The *in vitro* induction of microtubers with the same developmental and structural features as tubers growing *in vivo*, may allow researchers to answer the questions regarding the basic features of the effect of salinity on microtuberization (Figures 2, 3 and 4).

Genetic diversity was studied on the basis of microtuberization using analysis of cluster among potato cultivars. Dendrogram using average linkage (between groups) indicated that all cultivars were divided to four major groups in saline and non-saline conditions. Under non-saline conditions, first group contains Araucana INTA, Marphona, Surena INTA, FLS-5, Agria and Ranger-russet; second group contains AGB-69-1, Loman and MEX-32; third group contains Aracy and the fourth group contains Maine-28 and American-INTA. Under saline conditions, the first group contains Surena INTA, American-INTA, MEX-32 and Maine-28; second group contains Aracy, Agria and Araucana INTA; third group contains Ranger-russet, Loman, FLS-5 and Marphona and the fourth group contains AGB-69-1.

This clustering indicated that there were differences among all cultivars under saline and non-saline conditions. Under non-saline conditions, commercial cultivars (Ranger-russet, Agria and Marphona) performed better than wild-type cultivars and were ranked higher, whereas under saline conditions the commercial cultivars performed worse and were ranked lower (Figures 5 and 6).

Phenotypic correlations among characters under saline and non-saline conditions indicated that under non-saline conditions, there were highly significant negative correlations at $P< 0.01$ between the number of

Figure 3. Microtuberization of marphona cultivar in non-saline conditions (0 m moll^{-1} NaCl).

Figure 4. Microtuberization of Marphona cultivar under saline conditions (75 m moll^{-1} NaCl).

microtubers and the mean of minimum microtuber diameter. There were highly significant positive correlations at P < 0.01 among microtuber fresh and dry weight and the mean number of eyes on large microtubers and between the mean number of eyes and the mean number of eyes on small microtubers and between the mean of microtuber diameter and the mean of maximum microtuber diameter. A significant positive correlation was observed at P < 0.05 between the mean number of eyes and the mean number of eyes on large microtubers. A significant negative correlation was observed at P< 0.05 between the number of microtubers and microtuber fresh weight and between the number of microtubers and microtuber dry weight. No significant correlation was observed among other traits at these levels of probability.

Under saline conditions, there were highly significant negative correlations at P < 0.01 between the number of microtubers and the microtuber fresh weight and between the number of microtubers and the microtuber dry weight. There were highly significant positive correlations at P < 0.01 between the microtuber fresh weight and the microtuber dry weight and also between the mean number of

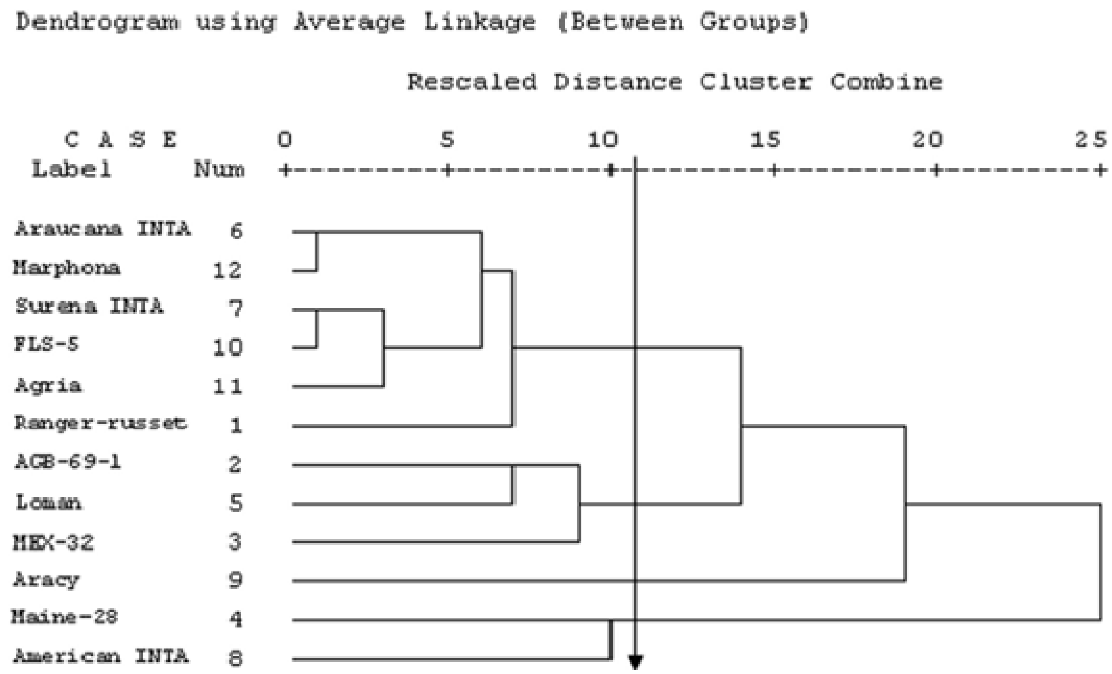

Figure 5. Analysis of cluster of cultivars under non-saline conditions.

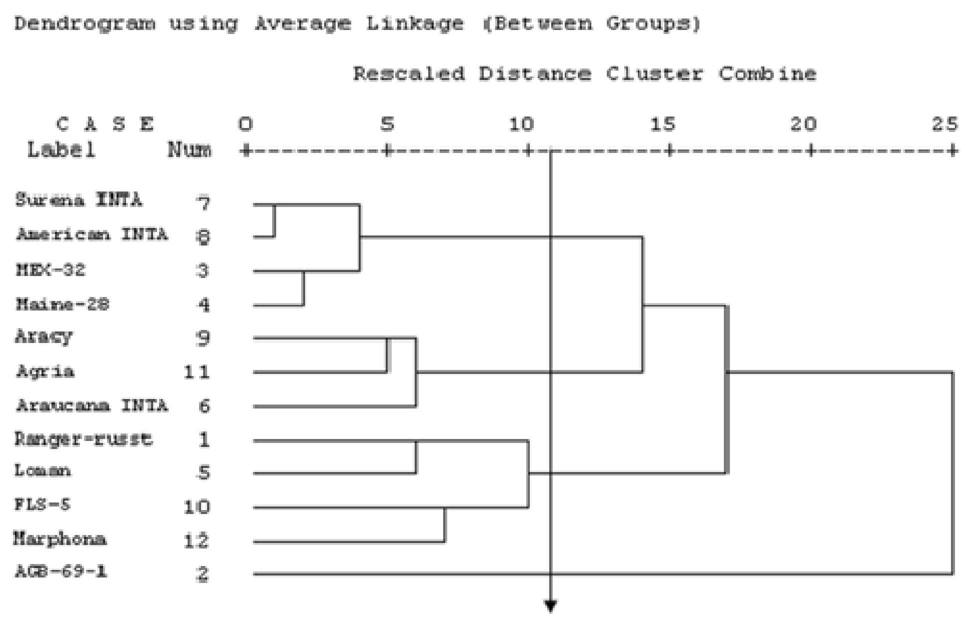

Figure 6. Analysis of cluster of cultivars under saline conditions.

eyes and the mean number of eyes on both large and small microtubers and between the mean of microtuber diameter and the mean of maximum microtuber diameter. Significant positive correlations were also observed at $P< 0.05$ between the microtuber fresh weight and the mean of maximum microtuber diameter and between the microtuber dry weight and the mean of maximum microtuber diameter and between the mean of microtuber diameter and the mean number of eyes on large microtubers and between the microtuber fresh weight and the mean of microtuber diameter and between the mean of maximum microtuber diameter and the mean number of eyes on large microtubers, and between the mean of minimum microtuber diameter and the mean number of eyes on small microtubers.

Phenotypic correlations among characters indicated that some associated factors correlating with each other contribute in the occurring of these characters.

REFERENCES

Ahmed R, Abdurah, Z (1982). Potato cultivation using sea water irrigation. J. Agro. Crop Sci. 151: 75-80.

Farhatullah R, Raziuddin M (2002). *In vitro* Effect of Salt on the Vigor of Potato (*Solanum tuberosm L*.) Plantlets. Biotechnol. 1(2-4): 73-77.

George R (1978). Vegetable Production in Iran. World Crops, Sept./ Oct. pp. 208-209.

Heur B, Nadler A (1998). Physiological response of potato plants to soil salinity and water deficit. Plant Sci. 137: 43-51.

Martinez CA, Maestri M, EG Lani (1996). *In vitro* salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. Plant Sci. 116: 177-184.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays of tobacco tissue cultures. Physiol. Planta. 15: 473-497.

Naik PS, Sarkar D, Gaur PC (1998). Yield components of potato microtubers *in vitro* production and field performance. Ann. Appl. Biol. 133(1): 91-99.

Nowak J, Colborne D (1989). *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. Am. Pot. J. 66: 35-45.

Sasikala DPP, Prasad PV (1994). Salinity effects on *in vitro* performance of some cultivars of potato. Braz. J. Plant Physiol. 6: 1-6.

Silva JAB, Otoni WC, Martinez CA, Dias LM, Silva MAP (2001). Microtuberization of Andean potato species (*Solanum spp*.) as affected by salinity. Sci. Hort. 89: 91–101

Turhan H (2005). Salinity response of transgenic potato genotypes expressing the oxalate oxidase gene. Turk. J. Agric. 29: 187-195.

Ulloa RM, MacIntosh GC, Melchiorre M, Mentaberry AN, Dallari P, Moriconi DN, Téllez-Iñón MT (1997). Protein kinase activity in different stages of potato (*Solanum tuberosum L*.) microtuberization. Plant Cell Rep. 16: 426-429.

Winicov I (1993). Gene expression in relation to salt tolerance. In: Basra, A.S. (Ed.) Stress-induced Gene Expression in Plants. Hardwood Acad. Publi., Switzerland pp. 61-130.

Zaman MS, Quraishi A, Hassan G, Raziuddin S, Kabir A, Gul N (2001). Meristem culture of potato (*Solanum tuberosum L*.) for production of virus-free plantlets. Online J. Biol. Sci. 1: 898-899.