Original Article

Horticultural Biotechnology Research 2015, 1: 11-15
http://scienceflora.org/journals/index.php/hbr/
doi: 10.5455/jhcbt.2015-06-03

Evaluation of electrophoretic patterns of soluble proteins in salt stressed potato callus

Setareh Forooghian*

Agriculture and Plant Breeding , Islamic Azad University of Borujerd Branch, Borujerd, Iran

Received: 30.06.2015
Revised: 27.07.2015
Accepted: 04.08.2015
Published: 04.08.2015

***Address for correspondence:**
Setareh Forooghian, Islamic Azad University of Borujerd Branch, Borujerd, Iran. E-mail: ahmadzadeh_ra@yahoo.com

ABSTRACT

Assessment of genetic diversity based on various markers plays a key role in crop breeding programs. In this study, protein patterns of callus, produced from three potato cultivar (Sante, Savalan, and Agria) and grown under salinity stress were investigated. Potato seedlings were generated *in vitro* and then explants were collected and transferred onto MS media containing 5 mg/l 2, 4-D, and 2 mg/l kinetin to callus induction. Callus was placed onto new MS media containing 0, 25, 50, 75, and 100 mM NaCl. A 1-month-old callus was evaluated in terms of protein patterns using vertical electrophoresis. The results indicated that potato cultivars are divided into two main groups. Sante cultivar grown under no salt stress was placed in one group, and Agria cultivar grown under different concentrations of NaCl put in a separate group. According to the results, most of protein bands were appeared in 6.5-29 kDa regions. In general, Savalan cultivar produced more protein bands than Sante or Agria.

KEY WORDS: Callus, electrophoresis, potato, salinity, tissue culture

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the most important non-cereal crop and fourth most important crop in the world. Potato is the most distributed crop after corn (Badr *et al*., 2001). Today, tissue culture methods are considered as powerful tools for the study of plants. One of the most important purposes of plant breeding is to produce salt-tolerant crop cultivars. It is believed that the use of tissue culture can help conventional breeding methods to increase plant tolerance to environmental stresses, particularly salt stress.

Selection of resistant plants to biotic and a biotic stresses can also occur at tissues and cells levels so that in tissue culture using isolated cells, callus, and differentiated tissues or obtained seedlings make us able to find resistance variables. Salinity decreases protein synthesis and increased protein hydrolysis in most plant species. Protein synthesis decreased in bean and soybean plants due to 72 mEq/l and 240 mEq/l NaCl, respectively. In a study, it was observed that salinity increased new protein synthesis and some amino acids such as alanine, aspartic acid, proline, lysine, threonine, leucine, isoleucine, tyrosine, serine, and valine were accumulated due to salt stress (Penuelas *et al*., 1997). It is suggested that catalase, peroxidase, and amylase activity would increase in response to mild salinity stress while decrease in response to severe salinity stress. Since diversity is the base of any selection, genetic selection needs to diversity. Expansion of selection, whether natural or unnatural, is associated with increasing genetic diversity in a certain population. Protein electrophoresis is suitable tools to identify and separate plant populations. Today, this method is widely used and is more effective and applicable for identifying species. Storage protein is having high polymorphism, are very stable. Environmental factors have little or no effect on mature seeds. Although these factors affect storage proteins amount, have minimum effect on its synthesis. Like isozymes, heredity is in the form of dominance. Most of the studies related to storage proteins are performed on those plants which their seeds are edible. Because of being incongruous in electrophoretic patterns and also intensity of bands, this method is more suitable for identifying those cultivars which are produced by cloning. In addition, self-pollinated crops which have little or no diversity are in the next priority. Electrophoretic studies of different populations have been based on non-enzymatic proteins and mainly storage proteins of seeds or tubers. The number of electrophoresis bands appeared in this method is usually more than isozymes patterns, and a

small amount of plant tissue is needed. Since storage proteins did not have enzyme activity, using common staining methods are efficient (Blackman and Payene, 1987). In order to analysis of electrophoretic patterns of storage proteins, polyacrylamide gel and isoelectric focusing methods is used. Acrylamide gels with high transparency and through densitometry rules allow us to do quantitative assays of proteins. Drying and keeping of these gels is easier than starchy gels (Douches, 1996). Identifying and classification of varieties is one of the most important purposes of plant breeding. Primary methods of potato classification were based on morphologic differences of tubers. The first electrophoretic study was done by Ali and Javad (2007) after that Rajapakes and Imai (1991) run potato extract on filter paper and identified six groups of soluble proteins (Rajapakes and Imai, 1991). Separated 59 potato genotypes using this methods followed by Burton (1989) identified more than 1000 potato genotypes. Ali and Javad (2007) have studied electrophoretic spectrum of potato proteins and found four-strong band near cathode called A, B, C and D so they divide potatoes into four main groups (Burton, 1989). In 1996, protein extracts of 12 potato genotypes were studied using same method and 8-10 bands for the first case and 28-32 bands for the second case were identified (Douches, 1996). In all genotypes two 23 and 25 kDa bands were observed possibly sporamine isomers bands. Another thick band was observed in 48 kDa region which was related to beta amylase enzyme. Ali and Javad (2007) have studied 25 potato cultivar using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. Amongst the potato cultivars, there were 7 very early mature, 6 early mature, 7 middle mature and 5 late mature. More than 75% of bands were appeared in 6-75 kDa regions. Svetlana and Matejova (2006) have studied 89 potato genotypes using morphological markers and simple sequence repeat (SSR) markers and then generated related dendrogram based on each marker.

Svetlana and Matejova (2006) showed that irrespective of high genetic diversity in both methods, there is a weak relation between dendrograms because of environmental effects on markers. Badr *et al*. (2001) investigated electrophoretic protein patterns of five potato genotypes using leaf, stem and tuber extracts and determined genetic distances. In addition to this, effectiveness of each protein source was evaluated. These results indicated that phylogenic relations are obtained from protein analysis which is expandable to physiologic and agronomic characteristics. Karuri *et al.* (2010) have stated that protein analysis method has high capability in identifying mutations. In 2010, 89 potato genotypes were studied using morphologic and SSR markers (Svetlana and Matejova, 2006) irrespective of high genetic diversity, in both methods, there was weak relation between dendrograms which is due to environmental effects. It has been reported that 40% of storage proteins of potato are patatine isomers which play a key role in tuber production and resistance to pests and pathogens (Zwartz, 1966).

Identify native genotypes with high yield capacity and resistant to environmental stress is one of the effective factors in economical independency of developing countries. Since native genotypes differ from one another in terms of yield capacity, it is necessary to know more about cellular and molecular characteristics of genotypes. There is no doubt that using compatible crops with environment and resistant to pest and diseases with high qualitative and quantitative yield, is a golden key to improve efficiency of primary inputs such as water and soil. Therefore, natural genetic sources which are considered as national wealth are the main genetic pools for plant breeders. In fact, genetic studies and genetic pools are the first loop in the chain of crop breeding. In other words, success in crop breeding programs needs to genetic diversity to select superior genotypes. Since water shortage and salinity are the main reasons for limited crop production and on the other hand, Iran is situated in arid and semiarid regions, the current study was aimed to investigate electrophoretic patterns of soluble proteins in three potato cultivars.

MATERIALS AND METHODS

In this study, protein patterns of callus of three potato cultivar (Sante, Savalan, and Agria) grown under salinity stress using vertical electrophoresis were investigated. Potato seedlings were produced *in vitro* and then explants were transferred onto MS media containing 5 mg/l 2, 4-D and 2 mg/l kinetin to callus induction. Callus was placed onto new MS media containing 0, 25, 50, 75 and 100 mM NaCl. One month old callus was collected and protein extraction was performed according to the modified method of Laemmli (1970). Briefly, callus was frozen at −20°C. After 24 h, callus was melted in room temperature for 1-h and powdered in mortar and pestle using liquid nitrogen. Three ml of extraction buffer was added. Extract was sucked up with a sampler and divided into microtubes for centrifuging at 15,000 rpm for 30 min at 4°C. The supernatant was collected at kept at −20°C. In order to run the samples

on the acrylamide gel (SDS-PAGE), microtubes were taken from freezer and left for 1-h at room temperature. Afterward, 17 µl of protein extract was added into new microtubes containing 17 µl of extraction buffer and mixed well. 30 µl of mixture was loaded into the wells made onto the gel. 20 µl ladder was loaded into lateral wells as marker. After running, the gel was placed into staining solution shaking for 12 h. After this time; the gel was washed into the second solution for 2 h. finally the gel was captured using scanner device.

Statistical analysis was performed using SPSS and NTSYS software.

RESULTS

Obtained results from electrophoresis indicated that average similarity of all samples based on similarity table is 27.5%. The minimum similarity (0%) was observed between Sante callus treated with 0 mM and 25 mM NaCl [Table 1]. The maximum similarity (57.7%) was observed between Sante callus treated with 78 and 100 mM NaCl and Savalan callus treated with 0 and 25 mM NaCl [Table 1]. In addition, total number of protein bands was 26. The most and the least bands were observed in Savalan callus treated with 0 mM NaCl and Sante callus treated with 0 mM NaCl, respectively. Protein bands were divided into three groups, light (6.5-29 kDa), middle (30-66 kDa) and heavy (67-205 kDa). The maximum protein band and protein diversity was found in the light region. Generated dendrogram indicated that samples divided into two main groups [Figure 1]. The first group was divided into two sub-groups [Figure 1]. Sante callus grown under 0 mM NaCl have placed in one group, and Agria callus grown under different concentrations of NaCl have placed in one different group. Similarly, the second group was divided into two sub-groups [Figure 1]. Sante and Savalan callus treated with different concentrations of NaCl have placed in one group and Savalan callus treated with 75 mM NaCl have placed in another group [Figure 1]. There was no

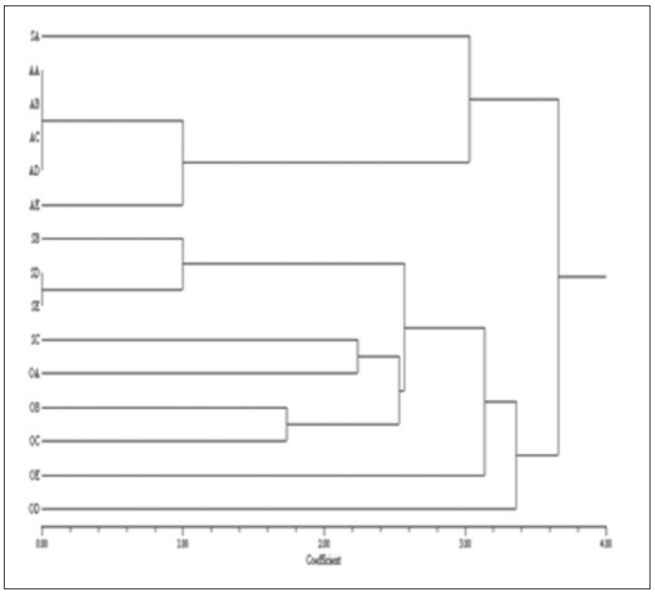

Figure 1: Dendrogram obtained from soluble protein analysis extracted from callus of three potato cultivars grown under salinity stress. SA: Sante treated with 0 mM NaCl, SB: Sante treated with 25 mM NaCl, SC: Sante treated with 50 mM NaCl, SD: Sante treated with 75 mM NaCl, SE: Sante treated with 100 mM NaCl, OA: Savalan treated with 0 mM NaCl, OB: Savalan treated with 25 mM NaCl, OC: Savalan treated with 50 mM NaCl, OD: Savalan treated with 75 mM NaCl, OE: Savalan treated with 100 mM NaCl, AA: Agria treated with 0 mM NaCl, AB: Agria treated with 25 mM NaCl, AC: Agria treated with 50 mM NaCl, AD: Agria treated with 75 mM NaCl, AE: Agria treated with 100 mM NaCl

Table 1: Genetic similarity of potato ctultivars using Jaccard's coefficient

Cultivars	SA	SB	SC	SD	SE	OA	OB	OC	OD	OE	AA	AB	AC	AD	AE
SA															
SB	0														
SC	0	50.0													
SD	0	53.8	50.0												
SE	0	53.8	50.0	57.7											
OA	0	46.2	57.7	50.0	50.0										
OB	0	46.2	50.0	50.0	50.0	57.7									
OC	0	34.6	42.3	38.5	38.5	46.2	50.0								
OD	0	29.9	38.5	26.9	26.9	38.5	34.6	34.6							
OE	0	42.3	50.0	42.3	42.3	46.2	42.3	38.5	38.5						
AA	0	23.1	23.1	23.1	23.1	23.1	23.1	15.4	11.5	30.8					
AB	0	23.1	23.1	23.1	23.1	23.1	23.1	15.4	11.5	30.8	34.6				
AC	0	23.1	23.1	23.1	23.1	23.1	23.1	15.4	11.5	30.8	34.6	34.6			
AD	0	23.1	23.1	23.1	23.1	23.1	23.1	15.4	11.5	30.8	34.6	34.6	34.6		
AE	0	23.1	23.1	23.1	23.1	23.1	26.9	19.2	11.5	30.8	34.6	34.6	34.6	34.6	

SA: Sante treated with 0 mM NaCl, SB: Sante treated with 25 mM NaCl, SC: Sante treated with 50 mM NaCl, SD: Sante treated with 75 mM NaCl, SE: Sante treated with 100 mM NaCl, OA: Savalan treated with 0 mM NaCl, OB: Savalan treated with 25 mM NaCl, OC: Savalan treated with 50 mM NaCl, OD: Savalan treated with 75 mM NaCl, OE: Savalan treated with 100 mM NaCl, AA: Agria treated with 0 mM NaCl, AB: Agria treated with 25 mM NaCl, AC: Agria treated with 50 mM NaCl, AD: Agria treated with 75 mM NaCl, AE: Agria treated with 100 mM NaCl

significant difference between Agria callus treated with 25, 50, and 75 mM NaCl. In Savalan callus, treatments differ from one another and the highest difference was observed in 100 mM NaCl treatment which was placed in a completely separated group. There was obvious difference between 0 mM NaCl and other salinity levels in Sante callus so that this treatment was placed in a separated group. Protein bands of three potato cultivars grown under different levels of NaCl are illustrated in [Figure 2].

DISCUSSION

According to the results, average similarity in all cultivars was 27.5%. Furthermore, the lowest similarity was observed in Sante callus grown under 0 and 25 mM NaCl while the maximum similarity (57.7%) was observed between Sante callus treated with 78 and 100 mM salinity and Savalan callus treated with 0 and 25 mM NaCl. The most and the least protein bands were observed in Savalan and Sante callus grown under 0 mM NaCl, respectively. The maximum protein band and protein diversity (6.5-29 kDa) was found in the light region. Generated dendrogram showed that cultivars are divided into two main groups as Savalan and Sante callus grown under different concentrations of NaCl have placed in one group and Agria callus grown under different concentrations of NaCl have placed in one separate group. Ali and Javad (2007) have reported that there are similar electrophoretic patterns in 75% of bands of 25 potato cultivars. According to (Badr *et al.*, 2001) results, obtained phylogenic relations from protein analysis are expandable to physiologic and agronomic characteristics. Svetlana and Matejova (2006) have studied 89 potato genotypes using morphological markers and SSR markers and then generated related dendrogram based on each marker. Canovas *et al*. (2004) showed that irrespective of high genetic diversity in both methods, there is weak relation between dendrograms because of environmental effects on markers. Bauw *et al*. (2006)studied protein extract of 12 potato cultivars using SDS-PAGE and showed that there were 8-10 bands for the first case and 28-32 bands for the second case. In all genotypes two 23 and 25 kDa bands were observed. Another thick band was observed in 48 kDa region which was related to beta amylase enzyme (Bauw *et al.*, 2006).

Ali and Javad (2007) have studied 25 potato cultivar using SDS-PAGE gel and showed that more than 75% of bands were appeared in 6-75 kDa region. Generally, Savalan cultivar showed that most protein bands compared with two other cultivars. In all cultivars, the most protein bands were appeared in 6.5-29 kDa region.

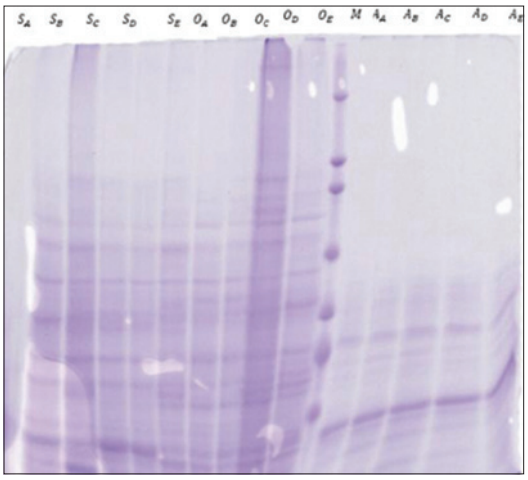

Figure 2: Protein bands obtained from electrophoresis of callus of three potato cultivars grown under salinity stress. SA: Sante treated with 0 mM NaCl, SB: Sante treated with 25 mM NaCl, SC: Sante treated with 50 mM NaCl, SD: Sante treated with 75 mM NaCl, SE: Sante treated with 100 mM NaCl, OA: Savalan treated with 0 mM NaCl, OB: Savalan treated with 25 mM NaCl, OC: Savalan treated with 50 mM NaCl, OD: Savalan treated with 75 mM NaCl, OE: Savalan treated with 100 mM NaCl, AA: Agria treated with 0 mM NaCl, AB: Agria treated with 25 mM NaCl, AC: Agria treated with 50 mM NaCl, AD: Agria treated with 75 mM NaCl, AE: Agria treated with 100 mM NaCl

REFERENCES

Ali A, Javad L. Study on variation of potato varieties using electrophoretic tuber storage proteins. Pak J Biol Sci 2007;10:3195-9.

Badr EA, Badawy OM, Hekal HA. Identification of five potato cultivars by isozyme patterns, in leaf and callus. Egypt J Genet Cytol 2001;30:61-74.

Bauw G, Nielsen HV, Emmetsen J. Patatins, Kuniz protease inhibitors and other major proteins in tuber of potato cv. Kuras 2006;15:3569-84.

Blackman J, Payene PI. Grain quality. In: Lupton FG, editor. Wheat Breeding. London: Chapman and Hall; 1987. p. 455-85.

Burton WG. The Patoto. 3rd ed. London: Chapman and Hall; 1989. p. 742.

Canovas FM, Dumas-Gaudot E, Recorbet G, Jorrin J, Mock HP, Rossignol M. Plant proteome analysis. Proteomics 2004;4:285-98.

Douches DS. Assessment of potato breeching progress in untied statues over the last century. Crop Sci 1996;36: 1544-1552.

Karuri HW, Ateka EM, Amata R, Nyende AB, Muigai AW, Mwasame E, *et al.* Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. Int J Agric Biol 2010;12:33-8.

Laemmli UK. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 1970;227:680-5.

Penuelas J, Isla R, Filella I, Araus JL. Visible and near-infrared reflectance assessment of salinity effects on barley. Crop Sci 1997;37:198-202.

Rajapakes DP, Imai T. Analysis of potato microtuber proteins by sodium dodecylsulfate polyacrylamide gel electrophoresis. Potato Res 1991;34:285-93.

Svetlana S, Matejova E. Characterisation of potato (*Solanum tuberosum* L.) varietiesby electrophoresis of tuber proteins. Czech J Genet Plant Breed 2006;4:142-6.

Zwartz JA. Potato varieties and their protein electropherogram characteristics. Eur Potato J 1966;9:111-28.