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MOLECULAR CHARACTERIZATION OF FOUR POTATO CULTIVARS (*SOLANUM TUBEROSUM* L.) COMMERCIALLY GROWN IN EGYPT

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ABSTRACT:

The present study aims to identify four different cultivars (i.e. Cara; Lady Rossita; Nicola and Spunta) of potatos (*Solanum tuberosum* L.) that are commonly planted in Egypt the tissue culture of each. In this respect, the tissue culture of each was established, while Spunta explants were considered best than others for invtro responses, when cultured on. Than different tissues (green leaves; calli and regenerated shoots) each cultiver used for biochemical and molecular analysis to measure genetic relationship among them when MS medium supplemented with 0.5mg/L NAA; 2.24mg/L 6-BAP and 8mg/L GA_3. Each cultivar differed for isoesterase and isoperoxidase electrophoretic patterns, as the highest differential expression of esterases and peroxidases were screened for regenerated shoots and callus tissues, respectively which may be due to a differential protein activation during with developing tissues. Furthermore, RAPD analysis fingerprints provided a number of useful DNA markers to distinguish and identify the four cultivars. According to the data of the six primers for all cultivars, the highest percentage of DNA polymorphism was screened for regenerated shoots. Such feature may be due to the somaclonal variation for *in vitro* culture, which is producing sequence dissimilarity among the original genotypes. Probably, due to this phenomenon, RAPD based dendrograms for green leaf; callus and regenerated shoot genomes revealed different genetic relationships among the four cultivars. Therefore, it may be concluded that plant leaves are preferred to design dendrograms of similarities and relationships among different cultivars. However, generally the somaclones may be a way of generating useful genetic variation and selection of desired traits for *in vitro* Plantlets.

INTRODUCTION:

The tetraploid (2n= 4x= 48) cultivated potato (*Solanum tuberosum* L.) belongs to family solanaceae is one, among most important vegetable crops where different techniques of tissue cultures are used. Isozymes analyses are useful for potato cultivar identification (Douches and Ludlam, 1991). Random amplified polymorphic DNA (RAPD) analysis has previously been used in genetic studies of potato for the identification of cultivares and clonal variations (Badr and Mabrouk, 2000) or somatic hybrids (Rasmussen and Rasmussen, 1995). It is also useful in the assessment of genetic diversity and relationships of cultivated and wild potato species. RAPD analysis is a fast, simple, cost-effective method and can give high resolution for DNA fingerprinting and genetic relationship studies in potato as well as in other crops (Isenegger *et al*., 2001). The Egyptian potato industry would benefit from more advanced methods that can facilitate rapid and accurate cultivar identification.

The present study was undertaken to identify a genetic relationship among four different cultivars of Egyptian potato (*Solanum tuberosum* L.). To achieve this purpose, optimum *in vitro* culture conditions were adapted and compared at callus formation and plant regeneration stages. In addition, some biochemical and molecular markers were determined in green leaves; calli and shoots of whole plantlets. Also, using molecular data, genetic similarity and relationships between four different cultivars were evaluated.

MATERIALS AND METHODS:

Potato cultivars: In the present study, Cara; Lady Rossita; Nicola and Spunta cultivars of

potato (*Solanum tuberosum* L.) were used (Fig 1), which were obtained from the Egyptian Company to Import and Store Potato.

Callus formation*:* Leaf disbis, of each potato cultivar were used as an explant for callus induction. The explants were prepared according to the method described by Badr *et. al.,* (2001). Then they were cultured on Murashige and Skoog medium (1962) supplemented with different hormones with various concentrations as given in Table-1.

Regeneration culture: Shoot induction in each potato cultivars was carried out when calli (five weeks of age) of each cultivar were transferred to MS medium (pH 5.7) containing 6BAP (2.24mg/L) and GA3 (10mg/L). the culture were incubated at 24ºC under white fluorescent lights (16-8 hours cycle of light and dark) for 3 months.

Isozyme analysis: Extracts were obtained by grinding each sample in small glass homogenizers with one volume of distilled water and centrifuged at 5000 rpm for 10 min at 4ºC. The supernatant was used for the enzymatic screening. Agar-starch-polyvinyl-pyrolidine (PVP) gel electrophoresis was carried out according to the procedure described by Sabrah and El-Metainy (1985). 10µl of each sample was absorbed into a small rectangle (4mm x 2mm) filter paper (Whatman No.1) and placed on the origin line of the gel. After storage for 30 min at 4ºC, filters were removed and a constant current for 2.5 hours at 4ºC was applied. Polymorphism of peroxidase and esterase were investigated according to Barta *et* al. (2003).

RAPD analysis: For each sample, genomic-DNA had isolated by using Mini DNA isolation Kit (Promega). The six random primers used for RAPDs are listed in Table-2. RAPD analysis had performed by Ready-to-Go Beads (Pharmacia). A total of 45 amplification cycles were performed. Each cycle consisted of 1min at 44ºC (denaturation); 1min at 35.5ºC (annealing) and 2min at 72ºC (extension). Following the final cycle, all amplified DNA products were completed with 10 min at 72ºC extension step as described by Demeke *et al.* (1993). Amplification products were separated on 1.8% agarose gels; stained with ethidium bromide; visualized with ultraviolet light and photographed. DNA fragment lengths were determined by comparisons with the DNA marker, which was run on each gel. The reproducible DNA bands from two runs were scored for their presence or absence in each genome. The Phoretix ID image analysis system (Phoretix International, London) was used to integrate the data of the RAPD bands.

Genetic relationships: Levels of marker polymorphism according to RAPD analysis were calculated. A similarity dendrograms for green leaves; calli and regenerated shoots of the different cultivars were recorded by using NTSYS (Numerical Taxonomic and Multivariate Analysis System software package, version 2.1, Applied Biostatistics Inc) as reported by Rohlf (2000).

RESULTS AND DISCUSSION:

Callus formation*:* In order to establish an efficient protocol for callus induction, Spunta explants were tested on MS medium containing different concentrations of the growth regulators. Table-3, illustrate that, the highest percentage of callus formation (92.36%) in the shortest time (21 days) was observed on MS medium containing 0.5mg/L NAA; 2.24mg/L 6-BAP and 8mg/L GA_3 (Medium number3). In addition, as shown in Table-4, the growth responses of callus formation on this medium varied among Cara; Lady Rossita; Nicola and Spunta cultivars. The percentages of callus formation ranged from 93.33% for Lady Rossita to 48.44% for Cara. Also, the duration of callus growth was between 21 and 29 days for Spunta and Cara cultivars, respectively.

Regeneration culture: A similar feature was reported for plant regeneration on MS medium

supplemented with 6-BAP and GA_3 (Table, 5 and Fig., 2). The percentages of regenerated shoots varied between 96% for Spunta cultivar and 60.42% for Nicola. Also, the time of growth ranged from 24 to 34 days for Lady Rossita and Cara cultivars, respectively. Similarly, many attempts have been carried out on callus induction and growth in potato (*Solanum tuberosum* L.). This has resulted in a range of protocols and procedures being established by researchers since tissue culture gained an importance in plant propagation; conservation and breeding. Yasmin *et al.* (2003) reported that callus induction and plant regeneration from explants require the presence of appropriate combinations and concentrations of plant growth regulators in the culture media. Moreover, they indicated that the growth responses somehow depend on the type of explants; media components; growth conditions and genotypes.

Isozyme analysis: For each developmental stage, the present data indicated that Spunta; Lady Rossita; Nicola and Cara cultivars differed in isoesterase and isoperoxidase electrophoretic patterns (Figures 3 and 4). In general, the total numbers of esterase bands (Table 6) were between one in Nicola and Lady-Rossita calli, and seven in Cara regenerated shoots. One or two cathodal esterases were expressed in some cultivars. Also, the anodal esterase bands ranged from one in Nicola and lady Rossita calli, to six in Cara regenerated shoots. The total number of isoperoxidase (Table 7) was between one band in Lady Rossita green leaves to eight bands in Spunta calli. The anodal bands ranged from one in green leaves of Cara and Lady Rossita, and regenerated shoots of Nicola to five bands in Cara regenerated shoots. The cathodal bands ranged from one in Spunta and Cara green leaves, and Nicola regenerated shoots to four in Spunta and Nicola calli. These data mean that, isozyme patterns give important results for the cultivar characterization and for the purposes of identification and verification of cultivar authenticity in potato trading (Barta *et al*, 2003). Similar conclusions were reported by for some potato cultivars (Badr *et al.*, 2001, Douches and Ludlam, 1991).

The obtained results for each cultivar showed polymorphic profiles for both isozyme systems at the different developmental stages. The highest differential expression of esterases and peroxidases was screened for regenerated shoots and callus tissues, respectively. The values of esterase bands were 7, 6, 6 and 3 for Cara, Lady Rossita, Nicola and Spunta shoots, respectively, with total of 22 isoesterases (Table 6). Comparatively, the total numbers of esterase isozymes were 13 and 8 for green leaves and calli, for four different cultivars. The values of isoperoxidases were 5, 5, 6 and 8 for Cara, Lady Rossita, Nicola and Spunta calli, with total of 24 bands (Table 7). While the total numbers of isoperoxidases were 9 and 18 for green leaves and regenerated shoots, of the four different cultivars. This polymorphism could be due to changes in protein expression during the plant regeneration process from leaf cells into a plantlets via cellular dedifferentiation and re-differentiation that are apparently programmed by certain genes (Castaneda and Mata, 2000). Also, the present polymorphism could be attributed due to somaclonal variations (Larkin and Scowcroft, 1981).

RAPD analysis: For the purpose, to estimate the DNA polymorphism among the four different cultivars as well as their genetic relationships, six random primers were used to amplify the genomes at the different developmental stages.

The present results showed variable numbers of polymorphic fragments per primer among Spunta; Lady Rossita; Nicola and Cara genomes (Figure 5 and Table 8). According to the results of the six primers, the highest numbers of polymorphic fragments were screened for Cara cultivar at regenerated shoots (22 bands) and green leaves (15 bands).

Also, the highest number of polymorphic amplified fragments at calli stage (22 bands) was indicated for Nicola genome. In addition, the database of RAPD fingerprints provided a number of useful DNA markers that are sufficiently specific to distinguish and identify the four cultivars. As shown in Table (9), the number of these DNA markers varied from one cultivar to another as well as also variant at different developmental stages. These results indicate that DNA fingerprinting using RAPD analysis successfully identified the four potato cultivars under study. The same molecular technique discriminated enormous numbers of potato cultivars that are grown in different countries all over the world (Bordallo *et al.*, 2004). Moreover, the present RAPD data suggests that the susceptibility to somaclonal variation could be related to genotype (Demeke *et al*., 1993). Likewise, differences in somaclonal variation incidence in potato cultivars were observed (Bordallo *et al.*, 2004).

For all cultivars under study, the highest percentage of DNA polymorphism (45.38%) was screened for regenerated shoots (Table 8). The same phenomenon was indicated for the percentage of the specific cultivar DNA markers reported for all cultivars at each developmental stage (Table 9). The highest numbers of specific DNA markers (19 fragments) were screened for regenerated shoots. Probably this feature is due to the accumulation of the somaclonal variations through the *in vitro* culture. The correlation between the culture time-length and the accumulation of chromosome variations was first documented in *Daucus carota* (Smith and Street, 1974).

Therefore, the accumulations of the somaclonal variations during the *in vitro* culture periods may have altered both callus and shoot genomes, producing somaclones (Larkin and Scowcroft, 1981). Consequently the genetic relationships among the original genotypes may have been modified. Therefore leaves seem to be best for characterization of different potato cultivars through RAPD analysis.

Genetic relationships: The dendrograms based on RAPD analysis of green leaves; calli and shoots genomes exhibited different degrees of similarity between Lady Rossita and Nicola cultivars (Figure 6). Also, variable genetic relationships of Cara and Spunta with both cultivars were identified. It may also be noted that primers which produced the highest number of polymorphic banding patterns are more useful for producing specific bands for their identification. Common bands help to establish genetic relationships.

Therefore, the accumulations of somaclonal variations during *in vitro* culture periods may have altered both callus and shoot genomes, producing somaclones (Larkin and Scowcroft, 1981). Consequently, the genetic relationships among the original genotypes may be modified due to in-vitro culturing. So, it may be concluded that plant leaves are preferred to design dendrograms of similarities and relationships among different cultivars.

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Table-1:Different Combinations & concen. of growth regulators used in callus induction medium.

Medium number	Concentration (mg/L)			
	2,4- D	NAA	6 BAP	GA_3
1	3	0.2	-	6
2	-	0.2	2.24	10
3	-	0.5	2.24	8

2, 4-D: 2, 4- Dichlorophenoxyacetic acid. **NAA:** α- Naphthalene acetic acid.
6 BAP: 6- Benzyl amino purine. **GA_3:** Gibberelic acid.

Table 2: The nucleotide sequences of primers used for RAPD analysis.

Primer Code	Sequence ($5^{/}\rightarrow3^{/}$)
AGERI-1	CGTCGCCCAT
AGERI-2	GGTAACCGTA
AGERI-3	CACAACGGGT
AGERI-4	TGGTCCTGGC
AGERI-5	GCCAGACAAG
AGERI-6	TGGTTCCCGA

Table 3: Growth responses of Spunta cultivar explants using three different protocols of callus induction media.

Medium number	Number of explant	Number of callus	Percentage of callus (%)	Time (days)
1	168	123	73.21	26
2	156	102	65.38	25
3	288	266	92.36	21

Table 4: Growth responses of the four different cultivars of potato on callus induction medium number3.

Cultivar	Number of explant	Number of callus	Percentage of callus (%)	Time (days)
Cara	384	186	48.44	29
Lady Rossita	360	336	93.33	23
Nicola	480	360	75.00	24
Spunta	288	266	92.36	21

Table 5: Regeneration responses of calli for the four different cultivars of potato on regeneration medium.

Cultivar	Number of callus	Number of plantlets	Percentage of plantlets (%)	Time (days)
Cara	160	103	64.38	34
Lady Rossita	240	225	93.75	24
Nicola	240	145	60.42	32
Spunta	200	192	96.00	25

Table 6: Frequency of esterase bands in green leaves; calli and regenerated shoots of the four different cultivars. C: Cara, L: Lady Rossita, N: Nicola and S: Spunta.

No. of bands	Green leaves				Calli				Regenerated shoots			
	C	L	N	S	C	L	N	S	C	L	N	S
1						1	1					
2								2				
3	3	3	3									3
4				4	4							
6										6	6	
7									7			
Total	13				8				22			

Table 7: Frequency of peroxidase bands in green leaves; calli and regenerated shoots of the four different cultivars. C: Cara; L: Lady Rossita; N: Nicola and S: Spunta.

No of bands	Green leaves				Calli				Regenerated shoots			
	C	L	N	S	C	L	N	S	C	L	N	S
1		1										
2	2		2								2	
4				4						4		
5					5	5						5
6							6					
7									7			
8								8				
Total	9				24				18			

Table 8: Summation numbers of polymorphic fragments for the four different cultivars of potato at different developmental stages. C: Cara; L: Lady Rossita; N: Nicola and S: Spunta.

Primer	Length of bands (Kb)	Total number of bands											
		Green leaves				Calli				Regenerated Shoots			
		C	L	N	S	C	L	N	S	C	L	N	S
1	1.862-0.376							5		7	1		6
2	2.650-0.279	8	8	3	3			3			4		
3	1.580-0.376						2			1	5		
4	0.890-0.376						3			1		2	3
5	2.500-0.430	7		6				10		10	5	1	2
6	1.660-0.376					3		4		3	3		
Total		15	8	9	3	3	5	22	0	22	18	3	11
Summation		35				30				54			
%		29.41				25.21				45.38			

Table 9: Total numbers of specific DNA markers per primer for each cultivar of potato at each developmental stage. C: Cara; L: Lady Rossita; N: Nicola and S: Spunta.

Cultivar/stage		Specific DNA markers						Total	Length of Fragments (Kb)
		Primer							
		1	2	3	4	5	6		
C	**Green leaves**		4			6		15	2.089-0.390
L			3						2.650-1.000
N						1			0.500
S			1						1.479
C	**Calli**						2	14	1.660, 0.376
L				2	2				0.890-0.376
N		3	1			2	2		1.585-0.376
S									-
C	**Regenerated Shoots**	3				1		19	1.750-0.550
L			3	4		2	1		1.585-0.279
N					1				0.750
S		2			2				1.580-0.376

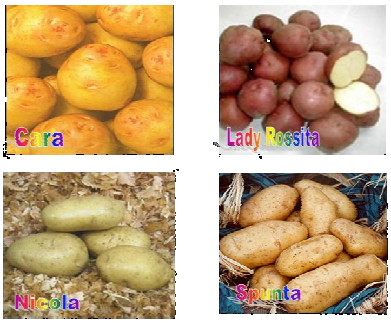

Fig. (1): Mini-tubers of four different Potato Cultivares

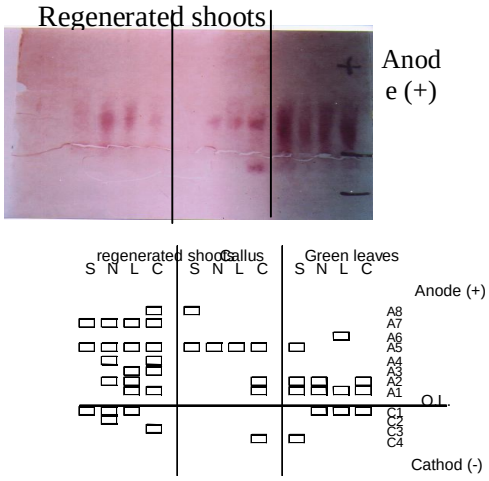

Figure 3: Photograph and zymogram showing, the electrophoretic profiles of esterase isozymes in green leaves; calli and regenerated shoots of the four different potato cultivars. (C: Cara; L: Lady Rossita; N: Nicola and S: Spunta.

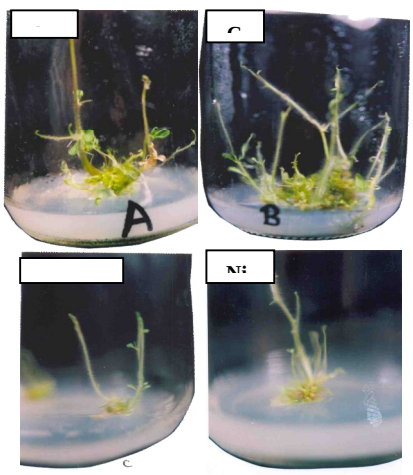

Figure-2: Plant regeneration on MS medium supplemented with 6-BAP and GA3. in 4-potato cultivars

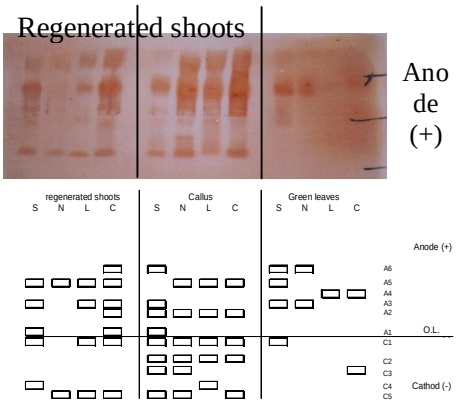

Figure 4: Photograph and zymogram illustrating the electrophoretic profiles of peroxidase isozymes in green leaves; calli and regenerated shoots of the four different potato cultivars. (C: Cara; L: Lady Rossita; N: Nicola and S: Spunta).

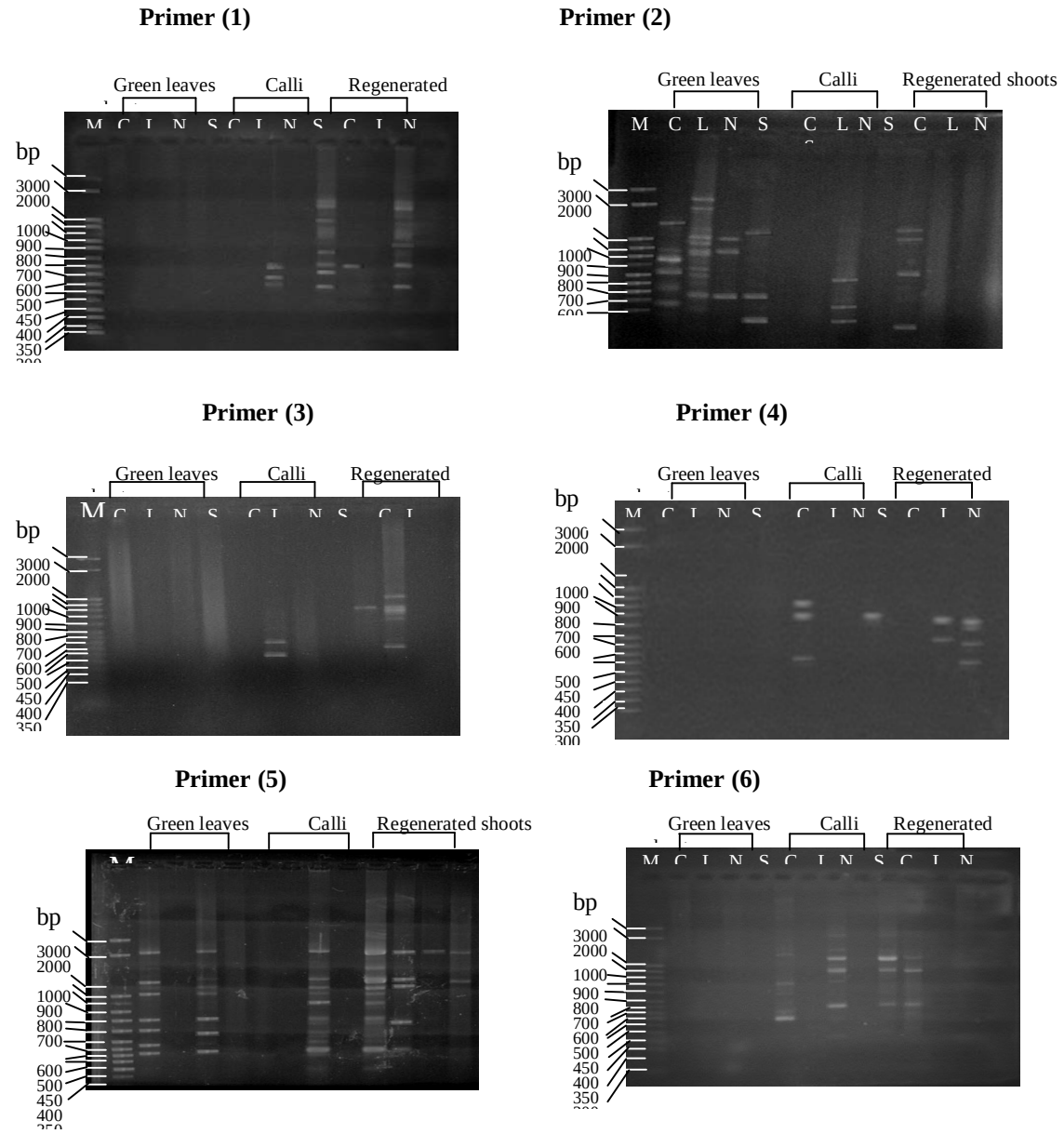

Figure 5: Photographs showing DNA-fingerprints of green leaves; calli and regenerated shoots of the four different cultivars using six random primers. C: Cara; L: Lady Rossita; N: Nicola; S: Spunta; M: DNA marker and bp: base pairs.

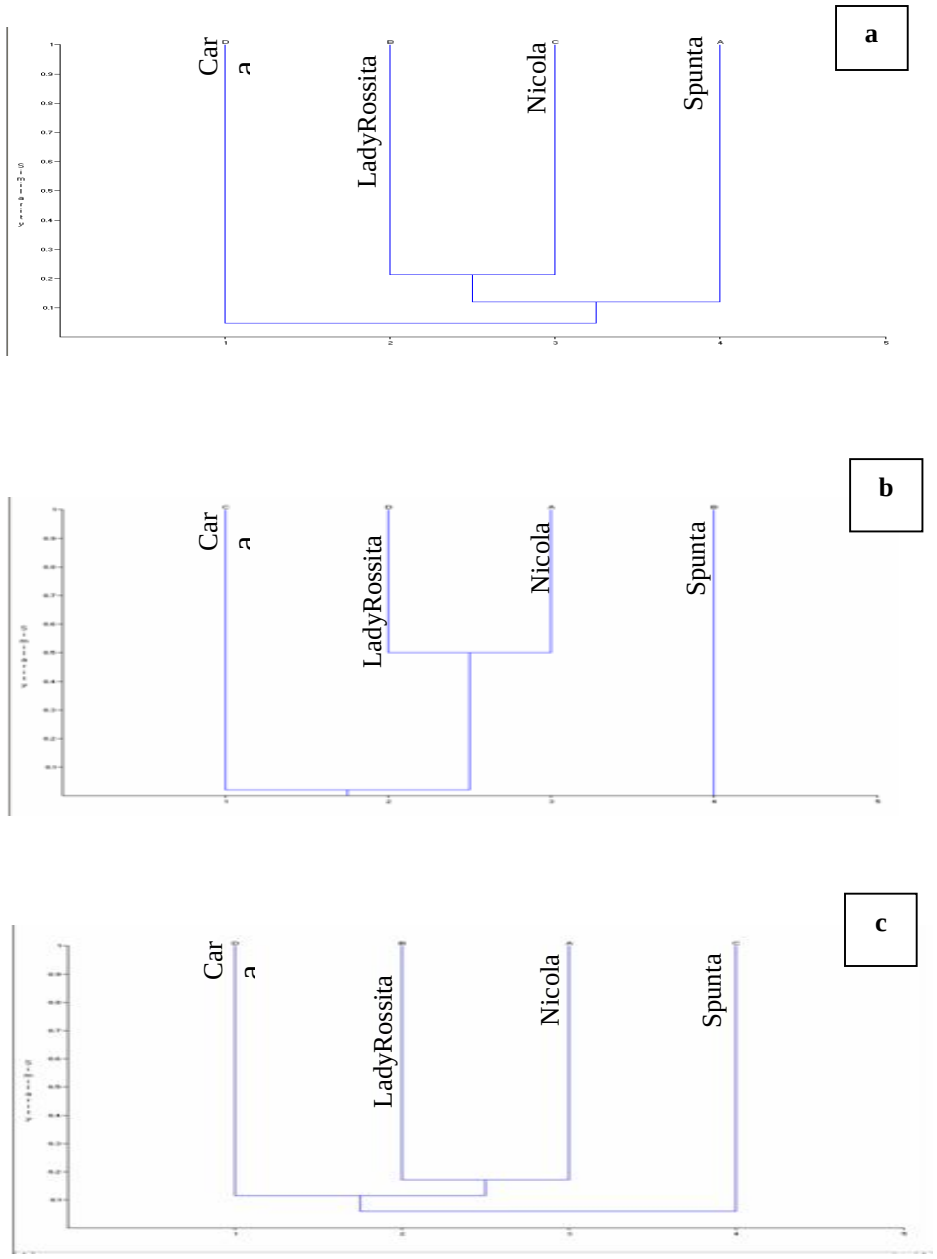

Figure 6: The genetic relationships among the four different cultivars of potato. The dendrograms are based on molecular data of green leaves (a); Calli (b) and regenerated shoots (c).

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