TRANSLATED FROM THE GREEK LANGUAGE

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FINAL REPORT

After application by the company **FARMAPLANT**, through its representative **Karipidis Lambrianos,** the Plant Genetic Improvement Laboratory of the University of Thessaly (Research Committee / Project code: 3185) conducted molecular genetic analyses in actinidia plant tissue samples of the commercial variety "Tsechelidis". The analyses aimed to develop the molecular profile (DNA fingerprinting) of said variety and compare it with the corresponding of the Hayward variety, in order to ascertain any differences sufficient to differentiate the compared varieties. We also conducted an examination of the fruits' morphological characteristics, weight and physicochemical properties, in comparison to the Hayward commercial variety.

(I) Molecular Analysis

The molecular analysis was conducted by PCR and was based on the analysis of small satellite regions (SSRs), which is regarded as the most reliable DNA analysis method in actinidia (Huang W.G., Cipriani G., Morgante M. and Testolin R.).

More specifically, for the analysis of the genomic DNA of the variety "cv. Tsechelidis" we used plant tissue samples from four reference genotypes:

a. Male clone used as pollinator for the Hayward variety.
b. Hayward variety.
c. Male clone originating from a Hayward variety seedling.
d. Commercial Variety "Tsechelidis"

DNA was isolated from a sample of young leaves (* 0.3 gr of original tissue) by modified CTAB. After the extraction of DNA, we proceeded to its quantitative determination by agarose electophoresis, using the plasmid Φx 174 as a standard control. The quantity and purity of the DAN were deemed satisfactory.

This was followed by a molecular genetic analysis by PRC and amplification of the small satellite DNA markers. In particular, we used 13 primers that amplified type AG/CT and AC/GT small satellite bibasic sequences.

More specifically, we used the primers: UDK 96-022, UDK 97-402, UDK 99-152, UDK 96-053, UDK 97-411, UDK 96-030, UDK, 96-001, UDK 96-037, UDK 96-034, UDK 99-168, UDK 96-092, UDK 97-406 and UDK 97-407 which were developed in the context of the international collaboration program INCO-DC (Italy – University of Udine, China – Chinese Agricultural University of Beijing, France – INRA, and Greece – University of Thessaly) and constitute the most reliable analysis markers in genetic material of *A. chinensis* variety actinidia.

The PRC amplification products were spaced in a 1.5mm thick 6% polyacrylamide gel layer, which was subjected to a voltage of 180 V for 1.5 hours. Nitric silver was used to achieve band appearance (Fig. 1- 5).

According to the specific PRC product spacing method, results showed that 7 out of 13 primers used were polymorphic, noting differences between the "Tsechelidis" and Hayward commercial varieties, verifying the severalty of the two examined genotypes.

Chart 1 below shows the differences of the two genotypes as regards to the presence or absence of same molecular weight alleles, based on the molecular markers presenting differences.

Figures 4 and 5 of the Appendix show characteristic and indisputable differences in the genetic composition of the two genotypes.

The data support that, based on molecular analysis using small satellite DNA markers (SSRs), the two genotypes are different as they present polymorphism in at least eight (8) alleles, as shown by the methodology followed and the molecular markers used.

Chart 1. Differences between the two genotypes based on the presence or absence of same molecular weight alleles. Presence is noted as (+) while absence as (-).

Primers / alleles	"cv. Tsechelidis"	"cv. Hayward"
99-152		
97-411	+	-
96-030		
96-037a	-	+
96-037b	+	-
96-034	+	-
96-092	+	-
97-406	-	+

APPENDIX

Pollinator	Hayward	Male Tsechelidis	Tsechelidis

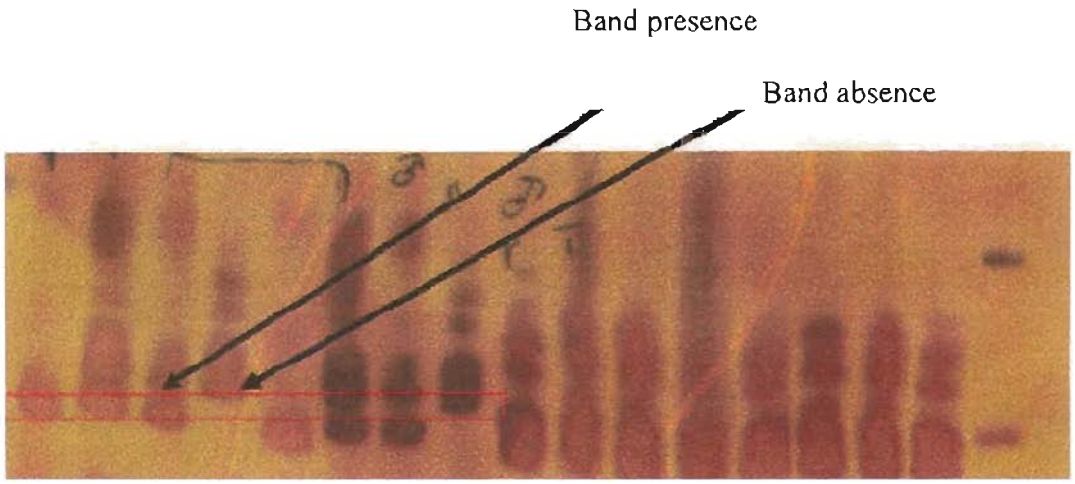

Fig. 4

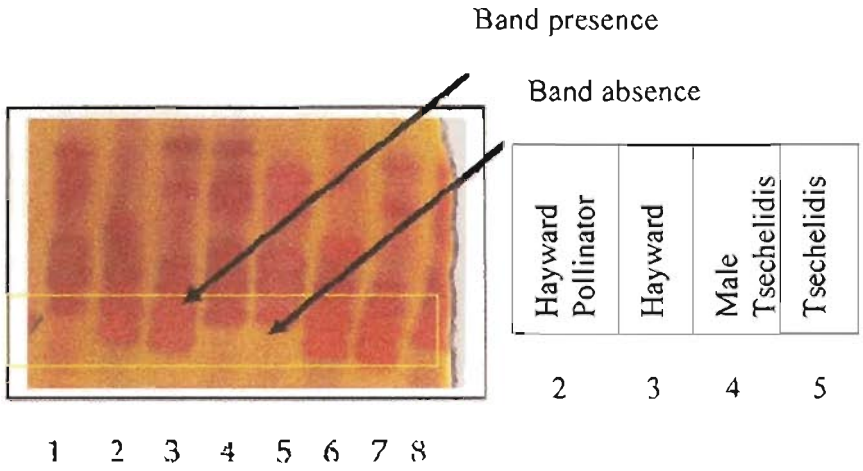

Fig. 5. DNA profile with repeated samples and using the molecular marker UDK 96-037

(II) Examination of the fruits' morphological and physicochemical characteristics

An examination of the fruits' morphological and physicochemical characteristics and weight was conducted in the two examined actinidia genotypes for two maturity stages and for two harvests (repetitions).

In particular, we measured the **fruits' shape**, based on length per fruit, maximum width per fruit, minimum width per fruit and calculated the maximum to minimum width ratio, the length to maximum width ratio and the length to minimum width ratio (**table 1**). The above parameters were only measured in unripe actinidia, as they could not be modified with maturation.

The results showed that the "Tsechelidis" variety actinidia presented higher values for all examined parameters (with the exception of the following parameters: maximum width and maximum to minimum width ratio, which were similar in both varieties) compared to Hayward variety actinidia. The similarities or differences between the two varieties reported above were identified for both harvests, while we could say that, in general, the shape of "Tsechelidis" variety actinidia is different to that of Hayward variety actinidia, mostly due to the larger length of the fruit. We did not observe any differences as to any of the above parameters between the 1st and 2nd harvest (**table 1**).

Table 1. Morphological characteristics of the actinidia commercial varieties Tsechelidis and Hayward.

Variety	Harvest	Length (cm)	Max. width (cm.)	Min. width (cm.)	Max/min width	Length/Max. width	Length/Min. width
Hayward	1st	7.40	5.65	4.98	1.137	1.314	1.488
	2nd	7.08	5.58	4.86	1.151	1.275	1.459
Tsechelidis	1st	7.96	5.71	5.15	1.114	1.397	1.550
	2nd	8.15	5.77	5.21	1.109	1.418	1.567
Significance							
Variety	***	NS	***	NS	***	***	
Harvest	NS	NS	NS	NS	NS	NS	
$LSD_{0.05}$	0.228	0.194	0.180	0.060	0.042	0.042	

The **weight per fruit** for cv. Tsechelidis was significantly greater that that of cv. Hayward for both harvests (mean 39%) in both unripe and ripe actinidia, while no differences are observed between the two harvests (**Table 2**). The Tsechelidis actinidia presented lower **pulp hardness** (they were softer) than Hayward actinidia (by one grade of the fruit maturity index) in both harvests, but only as regards to unripe fruit. In addition, pulp hardness was smaller during the 2nd harvest compared to the1st, in only the Tsechelidis variety.

Pulp hue presented significant differences between the two harvests in both unripe and ripe fruit, while smaller differences were noted between the two varieties. In summary, Tsechelidis actinidia presented a darker hue and pulp hue angle compared to Hayward actinidia at both maturity stages and for both harvests. Pulp hue in the 2nd harvest was slightly darker and less green (based on a* markers and Chroma) compared to the 1st harvest (**Table 2**).

Table 2. Weight, hardness and pulp hue characteristics for actinidia of the Tsechelidis and Hayward varieties.

Variety	Harvest	Maturity	Fruit weight (g)	Pulp hardness (kgF)	Pulp L*	Pulp a*	Pulp b*	Pulp C*	Pulp hue (°)
Hayward	1st	Unripe	136.5	8.30	65.1	-10.77	41.9	43.3	104.4
		Ripe	116.8	0.84	58.1	-8.38	35.9	36.9	103.1
	2nd	Unripe	126.2	8.26	63.8	-10.08	41.6	42.8	103.6
		Ripe	127.4	0.40	56.2	-7.66	31.9	32.8	103.5
Tsechelidis	1st	Unripe	178.2	6.48	60.4	-10.72	41.9	43.3	104.4
		Ripe	166.5	0.48	53.3	-7.61	35.2	36.0	102.2
	2nd	Unripe	189.9	5.69	60.6	-10.89	42.2	43.6	104.5
		Ripe	169.0	0.32	50.6	-7.03	31.5	32.3	102.6
Significance									
Variety	***	***	***	NS	NS	NS	*		
Harvest	NS	**	**	***	***	***	NS		
Maturity	***	***	***	***	***	***	***		
$LSD_{0.05}$	10.5	0.45	1.93	0.435	0.98	1.01	0.54		

Tsechelidis actinidia presented higher **soluble solids concentrations**, compared to Hayward **actinidia** (in both harvests and maturity stages). The two harvests presented no difference in soluble solids concentrations, as the fruit did not accumulate significant sugars or hydrolyze stored amylum in the 10 days between the two harvests. We should note that ripe Hayward actinidia presented SS (soluble solids) of approximately 14%, which means the fruit was of satisfactory quality, while ripe Tsechelidis actinidia presented SS of over 15%, which means the fruit was of high organoleptic quality (**Table 3**).

Tsechelidis actinidia presented higher **acidity** compared to the Hayward variety (in both harvests and maturity stages). Additionally, the acidity increased in ripe actinidia compared to unripe actinidia (only for the 1st harvest, and for both varieties) (**Table 3**). The Tsechelidis variety had higher total **phenolics** (a marker of human nutritional value) compared to the Hayward variety, in both harvests and maturity stages. The two harvests presented no difference in total phenolics concentration, which decreased as the fruit ripened (**Table 3**). This change was less prominent in the Tsechelidis variety, leading to an increased difference between the two varieties through time.

Tsechelidis actinidia displayed a higher **total conductivity** (a marker for the concentration of osmotically active organic and inorganic substances in the cellular humor, compared to the Hayward variety (in only unripe actinidia of the first harvest). No constant differences in total conductivity were noted between the two harvests and between ripe and unripe fruit (**Table 3**). Tsechelidis actinidia displayed a higher **special conductivity** (a marker for the fruits' maturity level) compared to the Hayward variety (in both harvests and maturity stages). As expected, special conductivity increased in the 2nd harvest, compared to the 1st (in both harvests and maturity stages). Additionally, special conductivity increased in ripe compared to unripe actinidia, as also expected (**Table 3**).

Finally, Tsechelidis actinidia displayed a higher **dry weight percentage** (a significant quality marker) compared to the Hayward variety. The dry weight percentage decreased in the 2nd harvest, compared to the 1st and in ripe compared to unripe actinidia (**Table 3**).

All above differences were small and mostly due to the low values of the ripe Hayward variety actinidia of the second harvest.

Table 3. Physicochemical characteristics of the Tsechelidis and Hayward varieties, in two maturity stages and harvests.

Variety	Harvest	Maturity	SS (%)	Acidity (%)	Total phenolics (mg%)	Total conductivity (μS)	Special conductivity	Dry weight (%)
Hayward	1st	Unripe	7.18	1.65	2.51	475	0.389	16.79
		Ripe	14.34	2.42	1.77	569	0.778	16.72
	2nd	Unripe	7.70	2.43	2.56	519	0.412	16.24
		Ripe	13.84	2.03	1.68	520	0.766	15.46
Tsechelidis	1st	Unripe	8.18	2.16	3.28	559	0.455	17.14
		Ripe	15.32	3.02	2.74	563	0.833	16.85
	2nd	Unripe	8.30	3.11	3.01	565	0.569	16.70
		Ripe	15.56	2.74	2.64	521	0.892	16.32
Significance								
Variety	***	***	***	**	***	*		
Harvest	NS	***	NS	NS	**	***		
Maturity	***	***	***	NS	***	*		
$LSD_{0.05}$	0.694	0.212	0.244	47.0	0.057	0.78		

In summary, we may say that the fruits of the Tsechelidis variety were of a different shape, with larger weight per fruit and were noted for their early maturity compared to the Hayward variety. In contrast, they presented minimum differences in pulp hue, matured faster at room temperature and were of a higher gustatory quality in both maturity stages (based on SS and acidity) compared to the Hayward variety.

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Certified true signature of A. Mavromatis
Volos, 26.04.2007
The secretary, *(illegible)* Tolia Marioli

Kind regards,
(signature)
Athanasios Mavromatis
Lecturer in Plant Genetic Improvement