

Apricot Genetics and Biotechnology in Romania

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ABSTRACT

The apricot genetic improvement programme performed in Romania between 1986-2006 included clearly formulated steps such as: wide species information, gene bank development based on worldwide exchange of the biological material, widened genetic variability through intra and interspecific hybrids, *in vitro* immature embryo research of interspecific hybrids from the *Prunus* genus, mutagenesis, genetic study of different valuable combinations in F₁, F₂, backcross generation, pollen grain study of various apricot phenotypes, molecular biology investigation, and tissue culture trials. Romania conserves a rich gene bank, including 655 apricot phenotypes originating from North America, Australia, Asia, and Europe. The genetic variability induced by conventional methods demonstrates that apricot-tree species still have genetic reserves for evolution. The reproductive barriers were overcome by the biotechnological methods applied after the hybridisation of ♀*Prunus armeniaca* × ♂*Prunus persica*, or ♀*Prunus persica* × ♂*Prunus armeniaca*, saving immature embryos often aborted in interspecific hybridisation due to nutritional non-compatibility. The genetic progress of the fruit quality characteristics resulted from backcross methods and physical mutagenesis by the mutagenic agent ⁶⁰Co 3000R. The electrophoretic investigation revealed peroxidase cryoresistance in the Comandor genitor. Strong shoots were obtained from MS culture medium supplied with 2.0 μM indole-3-butyric acid (IBA) + 2.0 μM benzylaminopurine (BAP) + 0.1 μM gibberellic acid (GA₃), using meristem tips as initial explants. Shoot elongation and caulogenesis were significantly improved in a medium prepared with depleted deuterium water instead of distilled water. Heterosis was independent of the culture medium composition. The 1983-2006 breeding programme validated the following varieties: 'Rareș', 'Valeria', 'Carmela', 'Viorica', 'Nicușor', 'Adina', 'Alexandru', 'Bucovina', 'Siret', 'Atractiv', 'Dacia', 'Excelsior', 'Favorit', 'Comandor', 'Olimp', 'Tudor', 'Traian', 'Cristal', 'Danubiu', 'Aurăș', 'Fortuna', 'Orizont', 'Amiral', 'Augustin'.

Keywords: *Armeniaca vulgaris*, genetic variability, heredity

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INTRODUCTION

Background to apricot culture and research

Known since ancient times, the apricot tree has been appreciated and grown, without particular attention, on mountain slopes in the temperate areas of Central Asia and China.

Apricot-tree culture is favourable in the latitudes of 35° and 43° North, and heights of 700-1,500 m, with rainfalls under 500 mm/year, and lowest temperatures of -33°C in the cold areas. The continental climate records no temperature fluctuations. In Central Asia and Asia Minor, the apricot tree grows at altitudes of 1,000-2,500 m (Lilleland 1935, Loschnig and Passeecker 1954).

Owing to its characteristics, the apricot tree is paramount in fruit-tree growing, and is renowned for the high food value of its fruit.

The apricot tree is particularly interesting from a fruit-tree growing viewpoint as it grows fast and yields fruit 3-5 years after planting; under favourable ecological conditions and adequate culture technologies, it can reach high yields each and every year (Bordeianu *et al.* 1967; Bălan 1991).

The studies performed on important European and American genetic apricot-tree collections, such as Yalta-Ukraine, Montpellier and Avignon-France, Bucharest and Constanța-Romania, Cacak-Yugoslavia, and Davis-USA, have emphasised a large range of phenotypes and genotypes originating from various geographic areas and recognizable, among others, by their ecological adaptability (Couranjou 1975; Bailey and Hough 1977; Layne 1980; Bălan 1991).

Many of the world cultivars come from local populations that are well adapted to their original places, although their pedigree is unknown. In his 1989 apricot-tree monography, F. Monastra reported that they held 15.7% of the total described cultivars (Strada *et al.* 1989).

All these apricot-tree phenotypes are a significant genetic inheritance of the entire world despite their impossibility of preservation in only one location and the requirements of registration and patenting which limit free access to genetic material of all the countries interested in developing apricot-tree growing.

Only 30 (23%) of the cultivars registered by the International Board for Plant Genetic Resources (IBPGR) are available in the commercial plantations of the main apricot-tree growing countries, i.e. France, Spain, Greece, Morocco (Audergon 1995).

Apricot culture and research in Romania

Romania has long been concerned with apricot-tree growing. In 1871, I. Hențescu published his work, "Pomology", providing recommendations for the growing of four apricot-tree varieties: 'Ananas', 'Nancy', 'Muscatello', 'Violeta' (the first two varieties still exist in the present fruit-tree collections).

By the end of the 19th century, there were several *ex-situ* apricot-tree collections in the nurseries of Bistrița Pietroasele and Vișani; some varieties are still grown today in some orchards in the apricot-tree growing areas: 'De Breda', 'Luiset', 'Rozal', 'Cea mai bună de Ungaria'/'Best of Hungary'.

In 1921, Nicola Krier created the first Romanian apricot-tree varieties, still sparsely grown: 'Dulci de Vișani'/'Sweet of Vișani', 'Târzii de București'/'Early of Bucharest' (Bordeianu *et al.* 1964).

After 1950, Nicolae Constantinescu and his collaborators, Vasile Cociu, Mircea Botez, Ecaterina Bumbac, set the basis of the first systematic research for the creation of apricot-tree varieties, implicitly concerned with the development of the genetic resources (Cociu 1981).

Vasile Cociu continued these studies on an international level between 1960-1980, his collaboration with Prof. Hough from Rutgers University being among the most memorable for the exchange of biological material and the development of the apricot-tree genetic resources.

After 1980, the activity was carried out even further by Viorica Bălan and her collaborators at SCDP Băneasa București, and Elena Topor and her collaborators at SCDP Valul lui Traian Constanța-Dobrogea, reaching from 62 apricot-tree phenotypes in 1980 to 518 phenotypes in 1990 and 655 phenotypes in 2003 (Cociu 1990; Bălan *et al.* 1993, 2006; Cociu 2006).

The preservation of the genetic resources resulted in studies on the apricot-tree genetic variability with particular focus on adaptability and quality, as well as the transmission of such hereditary traits as: flowering period and fruit maturation, fruit shape, soluble dry matter content, vitamin C content, fruit acidity, skin and pulp colour, resistance to frost and diseases, as well as the substantiation of some genetic-based improvement strategies (Bălan 1993).

The improvement methods were diversified, resulting in complex hybridisation between the best hybrids F₁, SIB and backcross hybridisation. Thus, a very large genetic diversity was created, allowing gene combinations and recombinations according to the aims of the studies.

The improvement strategies were based on the objectives specific to every new phase, in close relation with the objectives set in the most important fruit-tree research centres in: France (Couranjou J, Audergon JM), Italy (Bassi D, Guerriero R), Greece (Karayannis I), Spain (Egea J, Burgos L and Dicenta F), Turkey (Gülcan R).

Summary of world apricot biotechnology advances

The *in vitro* micropropagation of the apricot can be an efficient method of obtaining healthy, pathogen-free – mainly to Sharka – uniform plantlets, in a controlled environment, as well as of *in vitro* conservation of the genetic resources. The review of apricot genetics and biotechnology in Romania presents the most significant results obtained by different Romanian research teams in comparison with other authors' relevant works.

In 1989, G. Marino obtained plant regeneration in *P. armeniaca* and, by continuing his research, in 1993 he reported the importance of the carbon source on the multiplication rate, concluding that sorbitol (6.0%) gave better results than sucrose (3.0%). Balla and Vertesy (1999) studied the micropropagation of some Hungarian apricot genotypes, in order to obtain virus-free plants and noticed the importance of IAA (indole acetic acid) in the shoot elongation process. Remarkable results in apricot micropropagation were obtained by Pérez-Tornero *et al.* (1999, 2000), who successfully induced culture initiation starting from apical meristems, cultivated on the basal medium QL (Quoirin and Lepoivre 1977) or modified WPM (Lloyd and McCown 1981) of different phytohormone concentrations, obtaining the best results on the mediums supplied with BA (N⁶-benzyladenine) (1.78-3.11 μM). They also noticed the frequent phenomenon of apical necrosis of the *in vitro* cultivated *Prunus*. In the same year, Pérez-Tornero *et al.* (2000b) communicated the effects of BA (2.22–2.66 μM) on improving caulogenesis and the stimulatory effects of IBA (indole-3-butyric acid) (29.53 μM) and NAA (α-naphthaleneacetic acid) (10.74 μM) on rhizogenesis. Pérez-Tornero *et al.* (2006) also studied the effect of the explant origin on the *in vitro* culture establishment in four cultivars, pointing out that even the axillary shoots were significantly more contaminated than the meristems, the time to produce elongated shoots was shorter and there were no differences in rooting ability between the shoots micropropagated from meristems and from axillary shoots. Koubouris and Vasilakakis (2006) studied the factors that affect the rapid proliferation and rooting in an apricot cultivar representative for Greece. In order to obtain healthy plants, they used Na-ceftotaxime, an antibiotic, in the culture medium. To improve shoot induction, every two weeks they used subcultures in a medium supplemented with 2.2 μM BA + 5.71 μM IAA, and for the optimum root induction they used 19.6 μM IBA. Studying the acclimatisation phase of *Prunus* sp. rootstocks, Rogalski

et al. (2003) pointed out the significant effect of the IBA treatment, genotype and genotype x IBA concentration interaction, on the survival rate in *P. armeniaca* 'Capdeboscq'.

In Romania, research on the *in vitro* culture of the apricot developed in the last ten years, focusing on cultivar and hybrid micropropagation (Butic-Keul *et al.* 2004; Corneanu *et al.* 2006), interspecific ($\text{♀}P. armeniaca \times \text{♂}P. persicum$, $\text{♀}P. persicum \times \text{♂}P. armeniaca$) immature embryo germination (Bălan *et al.* 1999), and rootstock micropropagation (Popa *et al.* 2005). Different culture mediums and phytohormone balances, the effects of some unconventional bioactive substances (deuterium-depleted water, magnetic fluids) were tested in order to increase micropropagation or rooting rate.

Deuterium-depleted water (DDW) is defined as having the deuterium content under 80 ppm, which, compared with natural water, has 144 ppm. Recent research points out that there is a correlation between the deuterium content in water and DNA degradation. Cell ageing is connected to the gradual accumulation of errors in DNA or the dysfunctional DNA repairing mechanisms (Goodall 2003). In *Robinia pseudoacacia* var. *oltenica*, the DDW concentration in the culture medium had a significant effect on the shoot elongation, as well as on the organogenesis processes (Corneanu *et al.* 2006). Similar results were obtained by Butnaru *et al.* (2001, 2004) in *Chrysanthemum indicum* and Radovet-Salinski *et al.* (2004) in *Coleus blumei*.

Magnetic fluids (MF) are ultrastable colloidal suspensions of ferro- and ferrimagnetic particles in different liquids (water, petroleum, oleic acid, vitamins, a.o.). They were initially used in physics and technics (aerospatial industry) and, after the year 1990, they were tested in biology and medicine. The stimulative effect of magnetic fluids on the development and almost on the *in vitro* rooting process was reported by Corneanu *et al.* (2000, 2004, 2006), Butnaru *et al.* (1999) and Minea *et al.* (2003) in many species of horticultural interest and, in the last years, also in woody species (*Robinia pseudoacacia*, *Prunus avium*).

This review is structured into two distinct sections: genetics of apricot tree in Romania and biotechnology of apricot tree in Romania.

GENETICS OF APRICOT TREES IN ROMANIA

This section presents the research methods employed by the authors of this review, as well as the results obtained from the studies conducted by the corresponding author and the interdisciplinary team, between 1980 and 2006, in accordance with the internationally acknowledged scientists and specialist literature in the field of apricot genetics.

The apricot genetics and breeding programme in Romania included: cytogenetics, wide information on the species, adaptability, genetic, physiological and biochemical characteristics and traits, productivity, resistance to stable diseases, initial genetic variability, preservation of genetic resources, selection of genitors, widening genetic variability through intra- and interspecificities, mutagenesis, cryoprotein studies and embryo collection, genetic studies on heredity types and the genetic mechanisms referring to cytoplasmic heredity, genetic transgression, heterosis effect, dominance of some characteristics, segregation in F_1 , F_2 progenies, backcross and V_2 mutants, study on microcultures and competitive cultures of the most valuable apricot elites, and the validation as varieties of the apricot trees that corresponded to the breeding objectives.

Biological material for the study of initial genetic variability, preservation of genetic resources, selection of genitors

Devoted to the principle of evolution and preservation of biodiversity, Romania conserves a rich gene bank in the main areas: The Research Station for fruit tree growing Băneasa-Bucharest, with 655 phenotypes, and The Research

Station for fruit tree growing Constanța with 471 phenotypes.

The apricot-tree phenotypes preserved derive from the following geographic areas: North America (New Jersey-65, Canada-58, California-74), Australia-6, Asia (China-23, Middle Asia-2, Armenia-2, Iran-2), Europe (Romania-257, France-25, Germany-4, Yugoslavia-10, Greece-3, The Czech Republic and Slovenia-14, CSI-3, Italy-32, Ukraine-10, Macedonia-52, Spain-2, Holland-2, Hungary-12, the Republic of Moldova-5, Bulgaria-2).

Biological material for the study of induced variability and various genetic mechanisms

The biological material for the study of induced variability and various genetic mechanisms, and for the selection of new phenotypes was obtained from intra- and interspecific hybridisation, auto-fecundation, backcrossing, physical and chemical mutagenesis, and *in vitro* cultures.

Hybridisation resulted in 9,000 intraspecific and 1,100 interspecific hybrids.

The genitors used were: 'Comandor' standard, V2- 56 'Comandor', 'Excelsior', 'Dacia', 'Sirena', 'Selena', 'Olimp', 'Sulina', 'Litoral', 77.3.52BV, 'Valeria', 'Rareș', 'Carmela', 'Viorica', 'Favorit', 'Mamaia', 'Roșii de Băneasa', 'Red of Băneasa', 'Sirena', 'Sulina', 'Patriarca temprano', 'Early Orange', 'Goldrich', 'Harcot', 'HW 407', 'Skaha', 'Sungold', 'Sunglo', 'Vivagold', 630203, 'Wenatchee', 'Blenryl', 'Harogem', 'Sundrop', 'Stella', 'Riland', 'Kinred', NJA 58, NJA 25, 'Goldrich', 'Ksongady Magyar Kayszi', 'Salah', B12/6, NJA13, 'Farmingdale', NJA 20, 'Steaua Roșie', 'Red Star', 'Cais trandafriu', 'Rosy Apricot-tree', Marculesti 19, B 28/2, CR 2-63, 'ReUmberto' F_2 , 83.15.23 BI The auto-fecundation of the phenotypes 'Comandor', 'Olimp', 'Selena', 'Sulina', 'Litoral' resulted in 15-82 C_1 descendant. Within hybrid families, 15-230 descendants F_2 were obtained. Backcrossing resulted in 120 descendants. Physical mutagenesis used the mutagenic agent $^{60}Co_{3000R}$, and resulted in 140 V_2 'Comandor' mutants. Chemical mutagenesis used the mutagenic agent hydroxyl amine, and resulted in 20 M_1 'Olimp' individuals. 1100 hybrids resulted from the *in vitro* saving of immature embryos.

Statistical data processing

The data resulted from the studies conducted below were statistically processed for the following characteristics: tree height, crown height, crown diameter on the row and perpendicular to the row, trunk diameter on the row and perpendicular to the row, trunk-section surface, the ramification angle of the mother branches, the length and number of the annual shootlets per one metre of mother branch, the number of foreseen shootlets per fruit tree, the length of the two-year old branches per one metre of mother branch, fruit cluster formation, leaf surface per offshoot, respiration and photosynthesis intensity, the attack degree of some specific diseases, the free and bound water content, the carbon hydrates content of one-year old shootlets (both in the dormant and vegetative stages), the percentage of damaged and dead buds, the biometric elements of the fruit, the soluble dry matter content, the vitamin C content.

As the studied quantitative characteristics were expected to have the highest phenotypical value, sampling (average samples) was performed on more than 10 and less than 50 individuals from each tested population. Research in the quantitative genetics and statistical analysis of fruit trees in the US (West Lafayette), cherry tree in California, apricot tree in France and Romania, peach tree in France, genetically resistant plants in France and Romania, and horticultural plants in Romania, had previously reported that this type of sampling was sufficient to provide objective proof: Crăciun (1981), Lapins (1983), Hansche *et al.* (1975), Ceapoiu (1968,1980), Couranjou (1975, 1989), Monet (1967, 1977), Monet and Bastard (1971, 1982), Pena (1986), Cociu and

Oprea (1989), Bălan (1991); Bălan *et al.* (2006).

The measurement tools and devices were unchanged for all the sampled individuals and samples.

To analyse the variability of the studied traits and characteristics, it was agreed, according to the genetic experimental techniques, that the $s\%$ value of 0-10 refers to low variability, the interval 10-20 refers to average variability, and values over 20 refer to high variability. Environmental variance (V_E), calculated according to the Nokorinthop formula, was used to emphasise the influence of the environment on the phenotype.

$V_E = 1/3(s^2P_1 + s^2P_2 + s^2F_1)$ is the average between the parents and the generation F_1 .

Genetic variance is the genotype variance, and was calculated according to the formula:

$V_G = V_P - V_E$, where V_P is total variance, or the F_2 variance.

Heritability was calculated according to the formula $h^2 = V_E/V_P$.

The difference between the parental arithmetic means and the arithmetic means of the descendants F_1 , F_2 , C_1 , V_2 and M_1 was calculated.

Genetic studies conducted on apricot in Romania

Apricot-tree cytogenetics

The chromosomal set known until the present is $2n = 16$ (Darlington 1945; Bălan 1991). **Fig. 1** shows the chromosomal set present in the mitotic metaphase of active apricot-tree rootlets of *Armeniaca vulgaris*, hybrid '88.4.11 BI'.

The meiotic divisions observed in several apricot-tree varieties and hybrids showed no anomaly, which reflects the safe descendant transmission of some parental traits, i.e. their genetic continuity. **Fig. 2** shows that the meiotic divisions (diakinesis to prophase 1, metaphase 1, anaphase 1,

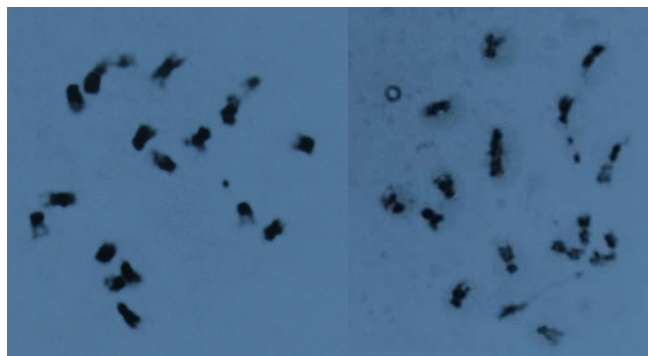


Fig. 1 Chromosomes in mitotic metaphase in apricot.

telophase 1 and metaphase 2, anelophase 2) were normal in the descendant hybrid '88.4.11 BI'. The display of chromosomes followed the Salesses method (1988).

Initial and induced variability, heredity type and genetic mechanisms of descendant trait transmission

The research methods and results refer to the initial and induced variability, the heredity type and the genetic mechanisms of descendant trait transmission: vegetative growth, morphological characteristics, certain physiological characteristics, frost resistance and wintering, behaviour under the main pathogen attacks – as elements of apricot-tree adaptability in the studied areas, production traits and fruit quality.

Variability of vegetative growth

The methods included in this sub-section had been previously established and used by the authors: Crăciun *et al.* (1978), Cociu (1969), Couranjou (1975), McLean and Rasmussen (1980), Renaud (1980), Bassi (1990), Bassi *et al.* (1995), Bălan (1991), Dejampour and Zeinalabedini (2006).

The following characteristics were recorded in the collection-preserved material, parents, hybrid descendants, CI crossbred descendants, backcross descendants, V_2 and M_1 mutants, both in the juvenile and maturity stages: tree height, crown height, crown diameter on the row, crown diameter perpendicular to the row, trunk diameter perpendicular to the row, trunk diameter on the row, trunk cross-section surface.

Tree height, crown height, crown diameter and crown volume

The traits – tree height, crown height, and crown diameter on the row and perpendicular to the row – were calculated with respect to their heritability.

The crown volume is characteristic for each phenotype, as the result of crown diameter and its height, calculated under conditions of free crown growth. The crown volume, measured in m^3 , was calculated based on the Sarger formula: $V = \frac{1}{2}(D+d) \times H \times 0.416$, where D = crown diameter on the row, d = crown diameter perpendicular to the row, and H = crown height. Calculations also included the initial and induced variability of the crown volume, and the correlation between its components; genetic analysis was performed on the crown shape, as well as the assessment of descendant selection of the apricot-trees that had a low crown volume in their juvenile phase and were likely to maintain it during their entire life.

The trunk diameters on the row and perpendicular to the

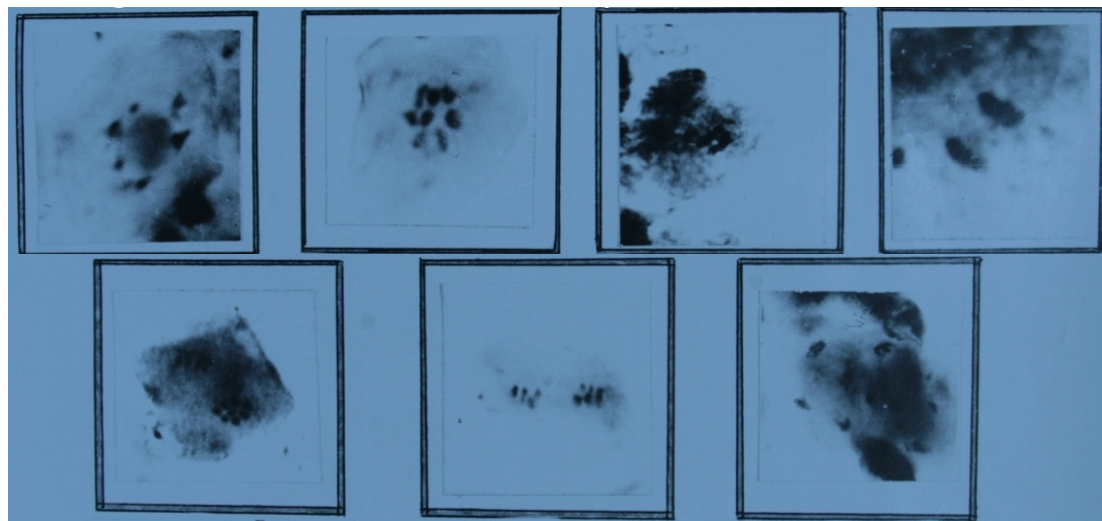


Fig. 2 Meiotic divisions in hybrid descendant 88.4.11.BI. Diakinesis to prophase 1, metaphase 1, anaphase 1, telophase 1 (top); Metaphase 2, anaphase 2, anelophase 2 (bottom).

Table 1 Heritability of tree height in apricot tree.

Hybrid family	No. of descendants	V _P M	V _E m/%	V _G m/%	H ²
Comandor × Excelsior	15	1.91	1.01/53	0.91/47	0.48
Excelsior × Comandor	18	2.05	1.06/52	0.99/48	0.48
Comandor × Dacia	22	3.43	1.73/51	1.70/49	0.49
Comandor × 77.3.52BV	12	1.66	0.94/57	0.72/43	0.43
Excelsior × Goldrich	50	1.91	1.0/53	0.91/47	0.48

row, measured in cm, were calculated in all fruit-trees, 50 cm above the soil surface.

The trunk-section surface was calculated using the following formula: $\pi \times 1/4(D+d)^2$. The growth rate of the trunk surface, i.e. the trunk thickness as a component of the fruit-tree vigour, was a selection criterion for the low-vigour phenotypes, together with the crown volume.

The following studies were conducted by Viorica Bălan, Elena Topor, Valerica Tudor and collaborators (1986-2006). They resulted in collection-preserved phenotypes in which the tree height varied between 3.0 m in the elite 77.3.52. BV-Romania, and 5.6 m in the following varieties: 'Cea mai bună de Ungaria'/'Best of Hungary'/'Hungary', 'Sulina'-Romania, and 'Sungold'-America. The amplitude of variation was 2.6 m, whereas the average height of all phenotypes was 4.8 m. 6% of the phenotypes were shorter than the average of all the preserved phenotypes. In the maturity period (seven years after planting), the amplitude of the initial variation in tree height allowed the selection of short genitors which were employed for the genetic study of this trait. The height variability of the hybrid descendants belonging to ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂Dacia, ♀Comandor × ♂77.3.52 BV was average, as the variability coefficient s% varied between limits (10.6-12.2) during the intense growth of the descendants, i.e. between years 2 and 4 when fruition started. The variation limits of the tree height in the fourth year of growth were 1.90 m in the descendant 83.8.16 BI (parents: ♀Comandor and ♂Dacia), and 2.15 m in the descendant 83.4.3 BI (parents: ♀Comandor and ♂77.3.52 BV). In the phenotypical illustration of the tree height, out of the total variance V_P, genetic variance V_G was between 43% and 49%, while environmental variance V_E was somewhat higher (between 51% and 57%). The heritability h² of the tree height varied between 0.43 and 0.49, which reflects the percentage of hybrid descendants inheriting the height of their genitors (Table 1). 30-50% were short-height hybrid descendants, and the maintenance of this trait during their ontogenetic evolution of growth and development (years 2-8), and the high value of the genetic variance V_G allowed the phenotype-based selection of apricot-tree elites that were shorter than their parents. On average, the hybrid generation was distinctly taller than the parents. Cytoplasmic heredity was identified in the tree height trait where the 'Excelsior' variety was used as maternal genitor. In the crossbred descendants C₁, the tree height variability was low in 'Sirena' C₁ (s% = 6.32) and 'Selena' C₁ (s% = 6.53), and average in 'Sulina' C₁ (s% = 12.26) and 'Litoral' C₁ (s% = 17.44). At a similar age, the crossbred descendants were shorter than the hybrid descendants, with the following limits: 0.40-1.45 m in year 2 compared with 1.20-1.90 m in the hybrid descendants, and 0.75-1.50 m in year 3 compared with 1.60-2.50 m in the same descendants. At a similar age (year 4), the average height of the mutants V₂ 'Comandor' (\bar{x} = 2.3 m) and M₁ 'Olimp' (\bar{x} = 2.55 m) was very significantly distinct from the standard phenotypes 'Comandor' (\bar{x} = 3.7 m) and 'Olimp' (\bar{x} = 3.80 m). Crossbreeding, physical (⁶⁰Co) and chemical (hydroxylamine) mutagenesis resulted in descendants shorter than their common ancestor, either 'Comandor' or 'Olimp'. There were insignificant differences in the average between the crossbred descendants C₁ 'Comandor' and V₂ 'Comandor', as well as between C₁ 'Olimp' and M₁ 'Olimp'.

In the collection-preserved phenotypes, the crown

heights varied between 2.4 and 4.8 m, the large diameter of the crown between 3.2 and 5.5 m, and the crown volume between 2.3 and 5.7 m³. In Romania, the shortest height (2.4 m) and width (3.2 m) of the crown was recorded in 77.3.52 BV. Also, narrow widths of the crowns were recorded in the following phenotypes: 'Dacia' (3.5 m) in Romania, 'Don Gaetano' (4.2 m) in Spain, and 'Canada 60012' (4.2 m), 'CR-2-63' (4.0 m), 'Manitoba' (4.2 m), 'Vivagold' (3.9 m), and 'Canada 6001' (4.2 m) in the US. In the hybrid descendants, the variations in crown height were low (s% = 9.61) in the ♀Comandor × ♂Excelsior family, or moderate (s% = 14-16.5) in the other hybrid families. In year 4, the crown height reached up to 2.3 m in 50-85% of descendants of the studied hybrid families, excepting those of the ♀Comandor × ♂77.3.52 BV family in which only 10% had this height while the rest were taller than 2.3 m. The crown volume ranged between narrow and average (10.8%) in the ♀Comandor × ♂Excelsior family, and large in the other families. Cytoplasmic heredity occurred for crown size and volume even if 'Excelsior' was the maternal genitor. The genetic variance V_G for crown height, large crown diameter, and narrow crown diameter was lower than the environmental variance V_E in all the hybrid families under study, i.e. between 28% and 48% of the total variance. The improvement value of crown height, large crown diameter, and narrow crown diameter was foreseen in 21-47% of cases, as heritability varied between 0.27-0.47 for crown height, 0.21-0.40 for large crown diameter, and 0.25-0.33 for narrow crown diameter. In the crossbred descendants C₁, the smallest crown size and volume were recorded in 'Sirena' C₁ while the highest were recorded in 'Olimp' C₁. The variation in crown height was low in 'Selena' C₁ (s% = 8.26), very high in 'Olimp' C₁ (s% = 98.4), and average in the following descendants: 'Sirena' C₁ (s% = 18.8), 'Comandor' C₁ (s% = 17.6), 'Litoral' C₁ (s% = 17.6). The large diameter of the crown recorded low variations in 'Selena' C₁ (s% = 6.45), and very high variations in 'Sirena' C₁ (s% = 34.4) and 'Sulina' C₁ (s% = 38.18). The narrow diameter of the crown showed high variability, as the variability coefficient s% was 25.2 in 'Sirena' C₁, and 45% in 'Comandor' C₁. For the narrow crown diameter, distinctly significant differences were determined between the mean of the crossbred descendants 'Olimp' C₁ and 'Sulina' C₁, and the standard phenotypes 'Olimp' and 'Sulina'. For the crown volume, the differences were distinctly significant between the crossbred phenotype 'Olimp' and the standard phenotype 'Olimp', and very distinctly significant between the crossbred descendants C₁ and the standard phenotypes 'Sirena', 'Selena', 'Comandor' and 'Litoral'. There was a large variation range in crown size and volume in the mutants V₂ 'Comandor' and M₁ 'Olimp'. In the relation between the standard phenotype 'Comandor' and the mutant phenotype V₂ 'Comandor', there were distinctly significant differences between the average crown heights, and very distinctly significant differences between the small diameters and volumes of the crown. The significant differences of the average crown size and volume between the standard phenotypes 'Comandor' and 'Olimp', and the crossbred phenotypes C₁ 'Comandor' and 'Olimp', as well as between the same standard phenotypes and the mutant phenotypes V₂ 'Comandor' and M₁ 'Olimp', proved the efficient crossbreeding and physical and chemical mutagenesis methods, resulting in the creation of new low-vigour phenotypes. The multiple regressions of the simultaneous influence on the

Table 2 Partial correlations between the crown height, large crown diameter, narrow crown diameter, and crown volume in the hybrid and crossbred descendants C₁ of apricot tree

Grade-2 correlations	Partial correlation coefficients (r)	Correlation signification highest r
rxzyw (H × D)	-0.97	000
rxzyw (H × d)	-0.81	0
rgzxw (D and d)	-1.10	000
rxwzy (H and V)	0.81	X
rywzx (D and V)	0.27	
rzwxy (d and V)	0.11	

x = crown height (H); y = large diameter of crown (D); z = narrow diameter of crown (d); w = crown volume (v) r (highest) 5% = 0.75; 1% = 0.87; 0.1% = 0.95.

three crown sizes of the hybrid, crossbred and mutant descendants emphasise the existence of a significant positive correlation between the crown height and the crown volume $r = 0.81$, and an insignificant correlation between the large diameter and the crown volume $r = 0.27$, and between the narrow diameter and the crown volume $r = 0.11$. A very significant negative correlation was evident between the crown height (H) and its large diameter (D), and the large crown diameter (D) and the narrow diameter (d) (Table 2).

Conclusions reveal that:

- For the height characteristics, $VG=1/2VP$ (genetic variance represents 1/2 of total variance) the methods employed were: controlled hybridisation, inbred by cross breeding, and mutations by physical and chemical gene mutation.

- For the crown shape characteristics, genetic recombination was involved by controlled hybridisation.

Genetic analysis of crown shape

The diallelic hybridisation between the oval-shaped and flat-shaped crown phenotypes resulted in descendants with three types of crown shape: oval, flat, and round. The analysis of the crown shape frequency in the hybrid descendants F₁ pointed that no segregation rapport can be established for this trait; consequently, the occurring non-parental flat shape was not the result of new gene combinations but of genetic recombinations. Thus, determinations regarding the recombination probability (p), the gametes (Os, as, Oa, ss) probability, phenotype frequency and, in the end, total crossingovers and non crossingovers were performed on the descendants from the following hybrid families: ♀Excelsior

(oval) × ♂Goldrich (flat), ♀Excelsior (oval) × ♂Comandor (flat), ♀Comandor (flat) × ♂Excelsior (oval), ♀Comandor (flat) × ♂Dacia (oval) ♀Comandor (flat) × ♂77.3.52 BV (oval) (Table 3). The analysis of the above data shows that the non crossover-determined parental phenotypes can occur with a frequency between 87% (♀Excelsior × ♂Comandor) and 97% (♀Excelsior × ♂Goldrich and ♀Comandor × ♂Dacia). The frequency of the non crossover-determined non-parental phenotypes can range from 3% (♀Excelsior × ♂Goldrich and ♀Comandor × ♂Dacia) to 13% (♀Excelsior × ♂Comandor).

Trunk diameter and trunk section surface

The trunk diameters on the row and perpendicular to the row, measured in cms, were calculated in all fruit-trees, 50 cm above the soil surface.

The trunk-section surface was calculated by using the following formula: $\pi \times 1/4(D+d)^2$ The growth rate of the trunk surface, i.e. trunk thickness as a component of the fruit-tree vigour, was a selection criterion for the low-vigour phenotypes, together with the crown volume

The results revealed that, in the collection-preserved phenotypes, the variation amplitude of the trunk section surface was higher in the Romanian varieties, compared with varieties from USA, Italy, France, Spain, and Asia Minor. The selection 77.3.52 BV recorded the smallest surface of the trunk section (69.4 cm²) whereas the Romanian variety ‘Litoral’ had the largest surface (147.3 cm²). The frequency polygon of the trunk surface variation represents a plurimodal distribution with a slightly negative asymmetry, which points to the heterogeneity of the biological material under study. In the four-year old hybrid descendants, the trunk section surface varied between 10.20-55.37 cm². The variation range was average for the trunks of the hybrid descendants of ♀Excelsior × ♂Comandor (s% = 10.7), and ♀Comandor × ♂Excelsior (s% = 15), and high in the descendants of ♀Excelsior × ♂Goldrich (s% = 29.09), ♀Comandor × ♂77.3.52 BV (s% = 33.9), and ♀Comandor × ♂Dacia (s% = 39.3). The configuration of the diagram and the regression line pointed that there was no actual correlation between the surface of the trunk section and the crown volume, as the correlation coefficient was $r = 0.58$. The crossbred descendants were characterised by significant differences in the average surface of the trunk cross-section compared with their ancestors: ‘Sirena’, ‘Selena’, ‘Olimp’, ‘Sulina’, ‘Comandor’, ‘Litoral’. The trunk section surface of the phenotype C₁ ‘Comandor’ was significantly different

Table 3 Genetic analysis of crown shape. (Viorica Bălan)

Hybrid family	Oval %	Round %	Flat %	p	Gametes (Os, as Oa, ss) probability	Phenotype frequency (Os, Oa, as, ss) %	Total cross overs %	Total non cross overs %
♀Excelsior oval × ♂Goldrich flat	77	19	4	0.04	Os/0.48 as/0.48 Oa/0.02 ss/0.02	Os/73 as/24 Oa/2 ss/1	3	97
♀Excelsior oval × ♂Comandor flat	28	56	16	0.16	Os/0.42 as/0.42 Oa/0.08 ss/0.08	Os/66 as/21 Oa/8.6 ss/4.4	13	87
♀Comandor flat × ♂Excelsior oval	50	40	10	0.1	Os/0.45 as/0.45 Oa/0.05 ss/0.05	Os/69 as/22 Oa/5 ss/4	9	91
♀Comandor flat × ♂Dacia oval	50	45.5	4.5	0.04	Os/0.48 as/0.48 Oa/0.02 ss/0.02	Os/73 as/24 Oa/2 ss/1	3	97
♀Comandor flat × ♂77.3.52 BV oval	71.4	14.3	14.3	0.14	Os/0.43 as/0.43 Oa/0.07 ss/0.07	Os/67.5 as/21.6 Oa/7.5 ss/3.4	11	89

from V₂ 'Comandor', i.e. it had lower vigour.

In conclusion, physical gene mutation and crossbreeding, induced inbreed and mutation were involved for fruit-tree vigour (crown shape and size, trunk-section surface).

Variability of some morphological characteristics

In this sub-chapter, we included methods that had been previously used by the authors: Decourtye (1967), Couranjou (1975), Bailey and Hough (1977), Barlow (1980), Hansche and Beres (1980), Sjukka-Lacza (1985), Bassi (1990), Bălan (1991), Bassi *et al.* (1995), Bălan (1999), Audergon *et al.* (2006), Bassi and Audergon (2006), Costes *et al.* (2006), Legave *et al.* (2006).

The research conducted by Viorica Bălan and collaborators in Romania is in accordance with the above-mentioned literature.

The morphological characteristics that may provide new phenotypes of higher values were studied with the aim of contributing to further practical and scientific information necessary to create new apricot-tree varieties that use solar energy more efficiently in order to obtain high top-quality yields and better adaptability to weather conditions.

The morphological characteristics studied by Viorica Bălan and collaborators were: the ramification angle of the mother branches, the length and number of the annual shootlets per one metre of mother branch, the number of foreseen shootlets per fruit-tree, the length of the two-year old branches per one metre of mother branch, fruit cluster formation, leaf surface per offshoot. Measurements were performed on groups of three fruit-trees belonging to each phenotype preserved in the collection, and on each individual and family in the case of the hybrid, mutant, C₁, F₂, and backcross populations.

The ramification angle of mother branches recorded high variations in the hybrid descendants, as the variability coefficient s% varied between 25.9% in the ♀Comandor × ♂77.3.52 BV family, and 57.5% in the ♀Excelsior × ♂Goldrich family. The adequate insertion angle of the mother branches (40-50°) was recorded in 25% of the ♀Excelsior × ♂Goldrich descendants, 35% of the ♀Comandor × ♂Dacia descendants, and in 40% of the ♀Comandor × ♂77.3.52 BV descendants.

The number of annual shootlets per one metre of mother branch varied widely in the hybrid descendants, the variation classes ranging from 10-95 in the first crown level, 12-99 in the second, and 12-108 in the third. Compared with the genitors, some of the descendants recorded less shootlets grown on one metre of mother branch, e.g. ♀Excelsior × ♂Goldrich and ♀Excelsior × ♂Comandor, while others had the same number as their genitors, e.g. in 40% of ♀Comandor × ♂Excelsior and in 15% of ♀Comandor × ♂77.3.52.BV; however, no descendant had more shootlets per one meter of mother branch. The length of the annual shootlets per one metre recorded average variations, as illustrated by the lowest limits of the variability coefficient, s% = 14 in the ♀Comandor × ♂77.3.52.BV descendants, and s% = 33 in the ♀Excelsior × ♂Comandor descendants. The number of foreseen shootlets (shootlet/shootlet) varied within 1-35 (in ♀Comandor × ♂Dacia) and 1-22 (in ♀Comandor × ♂77.3.52.BV). The induced variability of the foreseen shootles number varied widely, irrespective of the level: lowest in the F₁ ♀Excelsior × ♂Comandor descendants (s% = 39), medium in the F₁ ♀Comandor × ♂Dacia descendants (s% = 31), or highest, in the F₁ ♀Excelsior × ♂Dacia descendants (s% = 39).

The flowering branches per one metre of mother branch recorded wide variations in number and length, both within the same family and between families. The variation classes for the number of flowering branches per one metre of mother branch ranged between 1 and 50, while the branch length was between 1 and 60 cm. The descendant transmission of the flowering branch type was studied, pointing to the transgressive heredity of the characteristic, which allowed the identification of some spur-branched hybrids F₁

(3-6 cm), particularly in the ♀Excelsior × ♂Goldrich and ♀Excelsior × ♂Comandor families. The cytoplasmic heredity of the maternal genitor 'Excelsior' was also emphasised for the descendant transmission of the flowering branch type.

The distance of the fruting branch insertion on the multiannual branches varied between 1 and 50. The heredity of the branch framework was transgressive, as the distance between the fruting branches was either longer or shorter in the descendants, compared with their parents. The standard distance between the branches was considered to be 5-10 cm.

The leaf surface per offshoot unit varied within 15-40 cm² in the hybrid descendants, whereas the variability coefficient ranged between 26.7 in the F₁ ♀Excelsior × ♂Goldrich descendants and 34.8 in the F₁ ♀Comandor × ♂Excelsior descendants.

The conclusions of this study reveal that:

- Very distinctly significant differences of the average leaf surface were recorded between the 'Excelsior' and 'Goldrich' parents, as well as between these parents and their F₁ descendants.

- Similar to the parents, the angle between the leaves and the horizontal line in the descendants F₁ of the 'Excelsior', 'Goldrich', 'Dacia', 77.3.52BV phenotypes was either 30-45° or 60-90°, without exceeding 90°, which proved optimum for the best capture of solar energy.

- The 'Excelsior' phenotype showed its cytoplasmic heredity in the descendant transmission of the leaf surface trait.

- Genetic transgression and cytoplasmic heredity were involved by controlled hybridisation in the May-bunch and spur-form fruit branches.

Apricot-tree adaptability

Flowering time, fruit maturity, and photosynthesis-respiration

The methods included in this sub-chapter followed the specific methods for this field, presented in specialist literature, such as: Couranjou (1975), Kuebemen *et al.* (1979), Hansen and Ryngo (1979), (Bailey *et al.* (1983), Chang (1984), Suranyi (1985), Dorobanțu *et al.* (1986), Cociu *et al.* (1985), Loukas and Pontikis (1985), Audergon *et al.* (1988), Manganaris (1989), Bassi (1990, 1999), Bălan (1991), Badenes *et al.* (2006), Burgos (1995), Geuna *et al.* (2006), Muleo *et al.* (2006), Paydas *et al.* (2006), Poessel *et al.* (2006), Struss *et al.* (2006), Topor and Burtoiu (2006).

Adaptability is the capacity of the body to provide normal functioning of the vital and reproducible processes under the action of the weather factors. In order to emphasise the adaptability level of the apricot-tree phenotypes, observations and determinations were performed at the beginning and end of both the flowering and fruit maturation phases, in order to measure: the intensity of respiration and photosynthesis, the two simultaneous and opposed metabolic chains, the behaviour under conditions of frost and wintering, and the attack of some specific diseases.

Flowering and fruit maturation. The temperature necessary for the optimum fruition phenophases was calculated by summing up the active temperatures over the biological threshold (+6.5°C). It was considered that flowering began when 10% of the flowers bloomed, and ended when 90% of the flowers senesced, whereas fruit maturation was recorded when 90% of the fruit had ripened. Determinations referred to the amplitude of variability, the heredity of flowering, and the maturation of the fruit.

Respiration and photosynthesis intensity were determined in the phenotypes selected as genitors and the hybrid descendants in 100 g plant material/hour, in two important periods of time: 15 May-15 June, the time of the most intense physiological processes; and 15-29 September, when these processes slowed down.

The intensity of photosynthesis was determined by the

Ivanov-Kosovici method based on the determination of the carbon dioxide amount absorbed by the plants during photosynthesis, given a certain air volume. The CO₂ amount was calculated according to the following formula: $if = [(a-b) \times f \times 0.9 \times 60] / (g \times t)$. The intensity of respiration was determined by the Boysen-Jensen formula based on the amount of CO₂ released through respiration by the plant material. The variation amplitude of these physiological characteristics was determined in the parental phenotypes and their hybrid descendants.

In this field, the results obtained by Viorica Bălan and collaborators in Romania showed that controlled hybridisation resulted in descendants F₁ that exceeded the variation limits of the parental phenotypes, reflecting the transgressive heredity of the flowering trait. The differences between the descendants F₁ resulted from the same parents (alternatively used as mother and father), which pointed to the cytoplasmic heredity of the standard phenotypes 'Comandor' and 'Excelsior'. The heredity of fruit maturation was illustrated by three cases: transgressive (7%) to very early (5-25 June), in the combination of ♀late maturation × ♂semi-early maturation in ♀Comandor × ♂Dacia; dominant for the early maturation of the father genitor, in the combination of ♀late maturation (August) × ♂very early maturation (June) in ♀Comandor × ♂77.3.52 BV; and intermediary, in the combination of ♀average maturation × ♂late maturation in ♀Comandor × ♂Excelsior and ♀Excelsior × ♂Comandor.

The photosynthesis-respiration balance, both during the intensive growth of the shootlets and fruit, 15 May-15 June, and during the inactive phase (quiescence), pointed to the normal metabolism of the accumulated organic matter available for the vital activities, which reflected the physiological adaptation of the phenotypes selected as genitors, as well as their descendants, in the areas under study, i.e. the Romanian Plain and Dobrogea.

The results led to the following conclusions:

- Genetic transgressions and cytoplasmic heredity were involved for the blooming time of apricot F₁ descendants.
- For fruit maturation, genetic transgressions, dominant and intermediary heredity was involved by controlled hybridisation and induced gene mutations by physical mutagenesis.

Behaviour under conditions of frost and wintering

These traits were analysed according to the methods employed by: Hatch and Walker (1969), Hansche *et al.* (1972), Richardson *et al.* (1974), Erez *et al.* (1979), Raming (1980), Gilreath and Bouchanam (1981), Giulivo *et al.* (1983), Guerriero *et al.* (1985), Bălan and Ivaşcu (1989), Guerriero *et al.* (2006), Pedryc *et al.* (2006), Rodrigo *et al.* (2006), Ruiz *et al.* (2006).

There are numerous elements illustrating the resistance or susceptibility of the collection-preserved apricot-tree phenotypes, genitors and descendants in relation to low winter temperatures and fluctuating temperatures recorded at the end of winter and in spring time. Out of these elements, the determinations referred to: the free and bound water content and the carbon hydrate content, both in the dormant and vegetation stages, cryosusceptibility of malate dehydrogenase and peroxidase in the buds that were naturally exposed to frost during winter, and the percentage of dead flower buds.

Initial and induced variabilities, the heredity and genetic mechanisms of this characteristic were emphasised.

The carbon hydrate content of one-year old shootlets, both in the dormant and vegetation stages, was based on the extraction of sugars by ethylic alcohol and perchloric acid, respectively, under established conditions; the sugars were subsequently treated with anthrone (a tricyclic aromatic hydrocarbon). The starch extract was measured by photocolourimetry.

The cryosusceptibility of malate dehydrogenase and the peroxidase isosimic spectrums were analysed by using phenotype buds that had been naturally exposed to frost (-16°C)

and temperature fluctuations (-6°C +16°C).

Acellular homogenates were prepared by manual grinding with quartz sand in 1 Nm Tris- dithiothreitol buffer to protect enzymatic activity. The supernatants centrifugated with 2.6 mg/ml total protein were extracted by vertical discontinuous electrophoresis on polyacrylamide gels. The enzymatic activities were emphasised by the formazan reaction for malate dehydrogenase using nitrotetrazolin-blue and oxidized nicotin aminodimelcatide (NAD) while a buffer mixture of benzenidyn 1 mg/ml and perchydrol 0.03% was used for peroxidase.

The results revealed that the starch content of the annual shootlets varied widely, as the variation coefficient s% ranged between 21.7 in the F₁ ♀Comandor × ♂Excelsior descendants, and 78.9 in the F₁ ♀Comandor × ♂77.3.52BV descendants in their dormant stage, and between 45.5 and 60.7 in their vegetation period. The variation limits of the starch amount were recorded in December-February, between 0.17-4.32 mg/100 g dry matter in the F₁ ♀Comandor × ♂Dacia descendants, and 0.30-6.0 in the F₁ ♀Comandor × ♂77.3.52.BV descendants, whereas in April-May the variations were 0.30-1.45 mg/100 g in the former, and 0.39-6.10 in the latter. The soluble carbon hydrate content varied widely, recording higher values than starch, i.e. 1.35-13.85 mg glucose/100 g dry matter, compared with 0.17-4.32 mg starch/100 g in the F₁ ♀Comandor × ♂Dacia descendants in December-February, and 4.36-11.95 mg glucose/100 g, compared with 0.30-1.45 mg starch/100 g in April-May. The correlation between the starch-carbon hydrate rapport and the frost resistance of the flowering buds was observed in the F₁ ♀Excelsior × ♂Goldrich descendants, where 8% recorded losses of only 20-30%, while the starch amount was 1.54 mg/100 g higher than the amount of soluble carbon hydrates. The bound water content was higher in the hybrid descendants F₁ than in their parents, e.g. 0.4-5.5 mg/100 g dry matter in the descendants F₁ of ♀Comandor × ♂Dacia, 0.3-3.50 mg/100 g in the genitor ♀Comandor, and 0.26-4.1 mg/100 g in the genitor ♂Dacia. The heterosis of the bound water content was determined in the descendants F₁, compared with their parents. The free water content of the descendants F₁ recorded low or average variations, both in the dormant stage and the vegetation period.

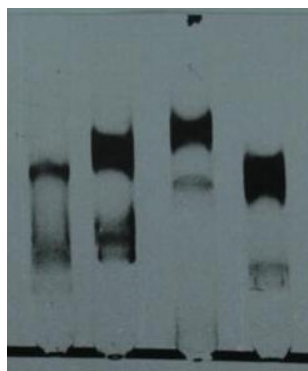


Fig. 3 Cryosusceptibility of cytoplasmic and mitochondrial dehydrogenase in genitor Comandor. Isozymic spectrums indicating decreasing activity in mitochondrial and cytoplasmic malate dehydrogenase.

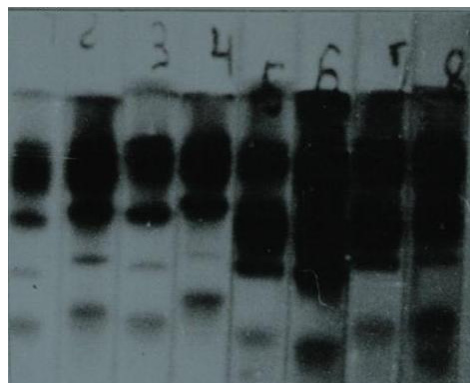


Fig. 4 Peroxidase cryoresistance in genitor Comandor. Isozymic spectrums indicating peroxidase activation.

The free water-bound water balance varied inversely, as the bound water level was higher than the free water level in the dormant stage, and lower in the vegetation period. The cytoplasmic and mitochondrial malate dehydrogenase had evident lower activity in the dead buds, compared with the living buds, in the genitors 'Comandor' and 77.3.52 BV, which emphasised their cryosusceptibility (Fig. 3). However, peroxidase manifested cryoresistance (Fig. 4), as the peroxidasic isosimic spectrum indicated no differences reflecting cryoinactivation, both in the living and the dead buds. Peroxidase cryoresistance and malate dehydrogenase cryosusceptibility were employed as control indicators for cryoresistance variation in the descendants F₁.

The conclusions resulted from the research data refer to the following:

- Heterosis of the content in carbon hydrates, free water and bound water present in the early shoots can be expected in descendants F₁.

- Transgressive heredity was revealed for the frost resistance traits of descendants F₁.

- There was a correlation between the peroxidase cryoresistance of the genitors and the frost resistance of descendants F₁.

Death of flower buds

Frost resistance of the flower buds is a biological characteristic influenced by such factors as: absolute temperature, temperature evolution from the end of the winter dormant stage to the flowering stage, and the physiological condition of the fruit-tree.

Specialist literature (Hatch and Walker 1969; Couvillon and Hendershott 1974; Erez and Lavee 1971; Spiegel-Roy and Alston 1979; Snir 1983; Paunovic and Plazinic 1976, 1983; Paunovic *et al.* 1983, 1985; Paunovic 1986; Scalabrelli and Couvillon 1985; Guerriero *et al.* 2006; Szalay *et al.* 2006) and personal research (Bălan and Ivaşcu 1989, 1995) show that sometimes the flower buds belonging to the same phenotype resist up to -25°C, while some other times they can die at -10°C.

The measurement of the damage caused to the flowering buds started in mid-January, after the temperatures decreased below -15°C, then followed in February-March, when temperatures fluctuated from (+) to (-), sometimes between +16°C and -16°C.

The percentage of damaged buds was determined, and the evaluation of resistance was based on marks ranging from 1 to 5 (Bălan 1991), as follows: 1 = highly resistant phenotypes (0-20% damaged buds), 2 = resistant phenotypes (20-25% damaged buds), 3 = slightly resistant phenotypes (51-70% damaged buds), 4 = sensitive phenotypes (71-80% damaged buds), 5 = highly sensitive phenotypes (81-90% damaged buds).

The results obtained by Viorica Bălan and collaborators emphasise that the flowering buds resistance to frost and wintering (-18°C) and to the temperature fluctuations recorded in February and March (-16°C to +16°C) was transgressively transmitted in descendance, as illustrated not only by the more resistant, but also by 10-25% more susceptible descendants than the genitors, e.g. in ♀Comandor × ♂Excelsior, ♀Excelsior × ♂Goldrich.

In conclusion, heterosis was found for the resistance of fruit buds F₁ to low winter temperatures (-18°C).

Behaviour under the attack of the main diseases

Research on the stable resistant diseases of the apricot tree is specific to various growing areas, as mentioned in specialist literature: Rishbeth (1964), Mănescu *et al.* (1975), Vanderzwet *et al.* (1975), Carter and Moller (1977), Klement (1977), Garrett (1979), Mircetich (1981), Ceapoiu and Negulescu (1983), Okie (1983), Rozsnyay (1983), Bălan *et al.* (1985), Morvan *et al.* (1985), Niyujto *et al.* (1985), Refatti (1985), El-Kady *et al.* (1986), Baicu and Săvescu (1986), Nemeth (1986), Dosba *et al.* (1988), Morvan (1988),

Karayannis (1988), Bălan *et al.* (1989), Crăciun and Crăciun (1989), Bassi (1990), Ionică *et al.* (1990), Bălan *et al.* (1995), Bălan and Stoian (1995), Bassi (1999), Bălan *et al.* (1999), Dicenta *et al.* (1999, 2006), Egea *et al.* (1999), Şesan and Oprea (1999), Karayannis *et al.* (2006), Krska *et al.* (2006), Nicotra *et al.* (2006), Oztürk *et al.* (2006), Romero *et al.* (2006), Trandafirescu and Teodorescu (2006).

Although literature mentions several apricot-damaging fungi, the most frequent in Romania are: *Monilinia laxa* (Aderh *et Ruhl*) Honey, *Stigminta carpophila* (lev) M. B. Ellis, and *Cytospora cincta* Sacc. producing important damage to all the apricot-tree growing areas.

Between 1982 and 2006, a team coordinated by Viorica Bălan conducted a research programme with respect to the genetics of resistance to stable diseases, aiming to create some phenotypes that are genetically resistant to high-frequency pathogen attack.

The particularity of this programme consisted in the absence of resistant genitors belonging to the wild species, spontaneous populations, or cultivated forms.

Therefore, the programme was started by providing natural infection conditions, i.e. plant treatments were stopped for three consecutive years. The selection lot consisted of 1600 F₁ descendants belonging to 64 combinations resulting from diallelic hybridisations of ancestral Romanian biotypes, complex hybridisation, backcrossing, hybridisation between geographically distant fruit trees, crossbreeding. Under strong infection conditions, selection was performed on the phenotypes resistant to the attack of the following pathogens: *Monilinia laxa* (Aderh *et Ruhl*) Honey, *Stigminta carpophila* (Lev) M. B. Ellis, and *Cytospora cincta* Sacc., mycoplasmas, bacteriosis, *Pseudomonas syringae* p.v. *syringae* van Hall, and viral diseases, particularly Plum-pox.

The selected phenotypes were vegetatively multiplied (20 trees from each phenotype), and then introduced into protected areas and screened by artificial inoculation.

Moreover, three apricot-trees from each collection-preserved phenotype, as well as each individual from the mutant, crossbred C₁ and F₂, and backcross populations, were artificially inoculated with the fungi *Monilinia laxa* and *Cytospora cincta* under orchard conditions, with minimum treatment applied to the collection and no other phytosanitary treatment. The phenotype variability of the reaction to pathogen attack was determined, and the resistant phenotypes were selected as genitors.

Diallelic hybridisation was performed between the phenotypes selected as resistant genitors, as well as between the resistant genitors and top-quality genitors. Determinations were performed on the descendants: induced genetic variability, genetic mechanisms, and the heredity types of resistance to stable diseases.

The attack produced by *Monilinia laxa* was observed on the branches after flowering, and the observations included the percentage of damaged branches out of 100 (F%) and the intensity (I%) of the disease on each branch, according to the assessment scale of 0-4 (Săvulescu and Săvulescu 1959). The degree of the attack was calculated according to the following formula: GA = (F × I)/100. Artificial inoculation was made by the virulent ML₂₇ strain of *Monilinia laxa* by spraying the sack-covered flowers with a spore suspension in sterile distilled water (concentration 10⁶ spores/ml).

The attack produced by the fungus *Cytospora cincta* was evaluated according to the drying level of the branches that had been artificially inoculated with fragments of fungus colonies by using two virulent strains: C₃₆ and C₄₁ grown on a CGA. medium. The inoculum was applied to scalpel-cut wounds made in October, and the evaluation of resistance to the action of the fungus was made by measuring the necrosis length starting from the infection point, as follows: 0-3 cm-resistant (R), 3.5-18 cm-medium resistant (MR), over 18 cm-susceptible (S) (Rozsnyai 1977).

The attack produced by the fungus *Stigminta carpophila* on leaves and fruit was determined by observing the frequency of the damaged organs out of a total of 100, and the attack intensity for each damaged organ by using the scale

of 0-6 (Baicu and Săvescu 1986). The degree of the attack was subsequently calculated according to the following formula: $GA = (F \times I) / 100$, where F = attack frequency and I = attack intensity.

The biological material was also tested for infection with such pathogens as *Apricot chlorotic leaf roll*, *Plum pox* (Sharka) and *Pseudomonas syringae* p.v. *syringae* van Hall; as the biotic factors were involved in apricot-tree damage, especially in complex actions together with micoses.

Micoplasmic infections were observed by optical microscopy, using the Diens method. Resistance to the attack of the bacterium *Pseudomonas syringae* p.v. *syringae* van Hall. was tested *in vitro*. Ten shootlets were sampled from each fruit tree belonging to a phenotype, as well as from each descendant, and subsequently inoculated with bacterial *Pseudomonas* suspension, a mixture of three strains: syringomicin-producing T₉₆, strong ice-nucleating T₁₃₂₈, and medium syringomicin-producing and medium ice-nucleating T₁₃₁₈ of 10⁹UFC titre.

To evaluate the attack of the virus *Plum pox*, samples of 150 leaves and 25 fruit were collected from each tree under study, and determinations were made for the intensity of the attack, the frequency of the attack, and the degree of the attack was calculated. For a better interpretation of the results, the entire biological material was subject to screening according to the degree of the attack and the results of the ELISA test. The frequency of the Sharka attack was calculated by using the formula $f\% = (n \times 100) / N$, where n = the number of attacked leaves or fruit, and N = the number of observed leaves and fruit. The intensity of the Sharka attack was calculated in order to analyse the spreading degree of the attack as the ratio between the area of the attacked leaves or fruit and the total area of the observed organs. The interpretation of the results was based on the scale of 0 to 6 classes equivalent to some percentage of the attack intensity (attack surface%/attack intensity class): (1-3)/1, (4-10)/2, (11-25)/3, (26-50)/4, (51-75)/5, (76-100)/6. The relative expression of the intensity of the *Plum pox* attack was calculated by the formula $I = [F(i \times f)] / n$, where F = attack frequency, i = class or percentage of the attacked area, f = number of attacked cases within the same class, and n = total number of attacked cases. The degree of the attack (GA%) illustrated the seriousness of the attack, and was determined according to the relation: $GA\% = (F \times I) / 100$.

The results show that the consecutive three-year absence of treatments and the conditions provided by the Bucharest area, where *Monilinia laxa* Aderh *et. Ruhl* Honey and *Stigmima carpophila* M. B. Ellis may produce over 70-80% damage, created favourable conditions for screening. Thus, out of 1600 descendants of 64 genomic families resulted from diallelic breeding between geographically distant partners, complex hybridisation, and backcrossing, only 13 descendants of nine genomic families were selected according to their field resistance to the two pathogens. The degree of attack (DA%) of the 13 descendants was limited to 2.5-7.5 in *Monilinia laxa*, and 1.8-7.5 in *Stigmima carpophila*. 'Marculesti 19' was the maternal genitor in three of the nine genomic families, and 'Re Umberto' was the maternal genitor in five families and the paternal genitor in one of the selected families. The selected elites were re-tested by artificial inoculation under glasshouse conditions, and no difference was observed, compared with their behaviour under natural infection conditions. The selection 83.15.23 (♀Re Umberto × ♂Timpurii de Chişinău/Early of Chişinău) was also resistant to inoculation based on fragments of *Cytospora cincta* colonies whereas selections 83.29.4 B1 and 83.29.3 B1 (♀Mr.19 × ♂CR5-180) showed average resistance. The selection 83.29.4B1 was highly resistant to pathogens and temperature fluctuations, and produced top-quality fruit; therefore, it was homologated in 1984 under the name of 'Dacia', and was protected by a patent. The phenotypes that resisted the attack of stable pathogens were the basis for the genetic study of resistance to diseases, as well as genitors in the genetic breeding programme for apricot trees. The study of the F₁ descendants

resultant from ♀resistant ('Dacia', 83.15.23B1, 77.3.52 BV) × ♂intermediary ('Comandor', 'Excelsior', 'Goldrich') showed high variability to the attack of the pathogens under study. The transgressive heredity of the reaction to the pathogenic action of the fungus *Stigmima carpophila* was determined in the following descendants F₁: ♀Comandor × ♂Excelsior, ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂Excelsior, ♀Early Orange × ♂Don Gaetano. The hybrid descendants F₁ ♀Excelsior × ♂Goldrich, ♀Comandor × ♂Dacia, of the ♀susceptible × ♂resistant type, and ♀Comandor × ♂77.3.52.BV of the ♀susceptible × ♂average resistant type, reacted to *Cytospora cincta* Sacc., manifested heterosis under conditions of inoculation with the virulent strains C₃₆ and C₄₁. The ⁶⁰Co physical mutagenesis, in a rate of 3000R, induced resistance to the fungus *Stigmima carpophila* in 33% of the total mutants V₂ Comandor.

The conclusions of the above results indicate that:

- Intermediate resistance to *Monilinia laxa* was involved by controlled hybridisation and physical gene mutation.
- Genetic transgression was induced by controlled hybridisation for resistance to *Stigmima carpophila*.
- Heterosis of *Cytospora cincta* resistance was induced by controlled hybridisation (VG>VP). Induced gene mutation was found for *Cytospora cincta* resistance and for the biochemical traits quality.
- Resistance to Sharka was analysed, resulting in the selection of resistant genitors, i.e. 'Stella', 'Early Orange', and NJA 42.

Yield characteristics

Yield level and regularity

Literature emphasises that apricot yield is still deficitary worldwide; therefore, yield characteristics are an important objective in the research programmes of the developing countries: Sansavini *et al.* (1974, 1988), Moore (1979), Friundlund (1979), Lespinasse *et al.* (1985), Crăciun (1987), Bălan (1991), Smith and Mollendorf (1999), Bertazzoli and Rivaroli (2006), Brunton *et al.* (2006), Ghelfi and Lucchi (2006), Mittermayer (2006), Olgun and Adanacioglu (2006), Albuquerque *et al.* (2006).

In Romania, Viorica Bălan and the research team analysed the fruit yield as a biological characteristic and, at the same time, as an indicator for the behaviour of the varieties under the given environmental conditions. Early fruiting, i.e. the fruiting time of the studied variants, was recorded for each tree belonging to the collection-preserved phenotypes, as well as for each descendant, and was genetically analysed.

High yields, i.e. marked 3 on a scale of 1-3, were recorded in 10-30% descendants F₁ of the following families: ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂77.3.52.BV, ♀Comandor × ♂Dacia, ♀Olimp × ♂CR₂₋₆₅. 30% mutants V₂ 'Comandor' were similar to the standard phenotype 'Comandor'.

Fruit quality

The complex physical and chemical characteristics of the apricots, such as the biometric elements and the biochemical components of quality, have been an important field of research for numerous specialists worldwide, among which: Couranjou (1975), Giulivo *et al.* (1983), Guerriero (1984), Cociu and Hough (1985), Margarian *et al.* (1986), Mehlenbacher and Hough (1986), Hansche (1986), Bassi (1990, 1999), Bassi and Negri (1991), Bălan (1995), Guerriero *et al.* (1995), Bălan *et al.* (1999), Bassi and Audergon (2006), Bălan *et al.* (2006a, 2006b), Benedikova (2006), Bureau *et al.* (2006), Ham (2006), Lachkar and Mlika (2006), Mencarelli *et al.* (2006), Pennone and Abbate (2006), Petrişor *et al.* (2006), Tzoneva and Tzonev (1999), Wang and Liu (2006).

The biometric elements and biochemical components of the fruit quality were determined for each individual studied,

and the data were statistically processed and analysed for: initial variability and the variability induced by hybridisation, crossbreeding, mutagenesis, F₂, backcrossing; the genetic mechanisms, and the heredity of the quality traits and characteristics; phenotypical correlations of some features referring to the fruit quality. A number of 30 fruit were sampled from each variant in order to determine its physical characteristics, and 2-3 kg were collected for the biochemical determination of the quality. The basic colour of the skin was evaluated by using the apricot-tree colour code devised by Ctifl France (Centre technique interprofessionnel des fruits et légumes) and adopted by the Romanian specialists.

Height (H), dorso-ventral diameter (D), and lateral diameter (d) were measured by the vernier caliper. The data obtained were statistically interpreted to emphasise variability and to calculate the shape index $I.F = H^2/D \times d$. When the index was higher than 1.0, the fruit was elongated whereas, when lower than 1.0, the fruit was either round or flat round. The average weight of the fruit was determined on 30 fruit sampled from each variant, and the results were statistically processed to obtain the initial and induced variabilities, and the descendant transmission of the respective trait. The pulp/stone ratio was determined by weighing the latter separately and relating the value to the total fruit weight, which resulted in pulp productivity.

The soluble dry matter content provided an overview of the content in carbon hydrates, organic acids, and mineral salts, determined by the Zeiss scaled refractometre. To avoid determination errors, fruit were sampled from all the variants after reaching the consumption maturity, the gathering time being based on the colour code and the Hunter lab measurements, originally correlated with the penetrometre-based determinations. The reducing sugar content was achieved by the Fehling-Soxhlet method, i.e. the polysugars and protein removal without converting saccharose into glucose and fructose. Vitamin C ascorbic acid, considered one of the most important vitamins for its catalytic functions and direct intervention in metabolism, was determined by the Thillmans method modified by 2-6 dichlorine-phenolindophenol. Titre was established by using ascorbic acid. Measurement was made in mg% of 100g fresh matter by the following formula: ascorbic acid (vitamin C) = $(V \times F \times 1000) / m \times mp$. The mean of the three determinations performed for each sample was statistically processed in

order to analyse the genetic variability and heredity of this important biochemical component of apricot quality.

The results point out that genetic variability was induced for the following fruit quality traits: average fruit weight (g) in the backcross descendants ♀Comandor × ♂F₁ ('Excelsior' × 'Comandor') s% = 25.42, and F₂ 'Olimp' s% = 21.80; titratable acidity-malic acid (g%) in the mutants V₂ 'Comandor' s% = 29 and F₂ 'Olimp' s% = 20.11; soluble dry matter (DM%) s% = 24.02, titratable acidity-malic acid (g%) s% = 46.06, ascorbic acid-vitamin C (mg.%) s% = 26.60 in the F₁ ♀Olimp × ♂NJA19 descendants.

The alternative use of the variety 'Comandor' as maternal and paternal genitor, in combination with the variety 'Excelsior', resulted in low variability in the former case, and average variability in the latter, which points to the cytoplasmic heredity of both partners. The study of the genetic progress achieved in the descendants F₁, F₂, backcross, mutants V₂, pointed to the statistically assured increase in the fruit quality traits, compared with one or both parents in the following cases (Table 4): a) soluble dry matter in the backcross generation ♀Comandor × ♂F₁ ('Excelsior' × 'Comandor'), mutants V₂ 'Comandor', and descendants F₁ ♀Olimp × ♂NJA19; b) ascorbic acid-vitamin C in the descendants F₂ 'Excelsior' × 'Comandor', mutants V₂ 'Comandor', and descendants F₂ 'Olimp'; c) average fruit weight in mutants V₂ Comandor. The values of soluble dry matter (DM%) of the standard phenotype 'Comandor' were compared with the backcross descendants 'Comandor' and mutants V₂ 'Comandor', emphasizing an increase from 15.6% in the standard phenotype to 18.46% in the backcross descendants, and 19.56% in the mutants V₂. An increase in soluble dry matter, compared with the standard phenotype 'Comandor', was also recorded in the descendants F₁ ♀Comandor × ♂Dacia, i.e. 16.29% on average. The mutants V₂ 'Comandor' recorded an increased content in ascorbic acid-vitamin C, as well as increased average fruit weight, which showed that physical mutagenesis was an efficient method of improving some fruit quality traits.

The analysis on the genetic mechanism of the fruit shape heredity in the descendants F₁ of five genomic families emphasised three types of gene actions: no descendent segregation, 3:1 segregation, and 1:1 segregation. The non-segregated monohybrid descendants were ♀Comandor × ♂Dacia elongated fruit Aa × round fruit aa, and ♀Comandor × ♂77.3.52 BV elongated fruit Aa × elongated fruit Aa.

Table 4 Genetic variability induction of some quality characteristics in the descendants F₁, F₂, backcross, mutants V₂, and signification of differences from parents or standard phenotype. (Viorica Bălan and Valerica Tudor)

Genitors/Descendants	Dry matter (DM%)	Malic acid (g%)	Vitamin C (mg%)	Fruit weight (g)
F ₁ ♀Excelsior × ♂Comandor	15.8	1.67	15.5	43.4
F ₁ -P ₁ × P ₂	-1.89	-0.04	2.54/**	11.16/000
F ₁ -P ₁	-4.0/000	0.45	1.58	11.16/000
F ₁ -P ₂	+0.21	-0.53	3.5/***	11.16/000
F ₁ ♀Comandor × ♂Excelsior	14.1	1.58	12.74	40.07
F ₁ -P ₁ × P ₂	-3.59/000	-0.13	0.22	14.93/000
F ₁ -P ₁	-1.49	-0.62	0.74	14.93/000
F ₁ -P ₂	-5.7/000	0.36	-1.18	14.93/000
F ₁ ♀Comandor × ♀Dacia	16.29	1.98	11.80	44.9
F ₁ -P ₁ × P ₂	+0.98	0.16	-1.40	26.1/000
F ₁ -P ₁	+1.68	0.22	-0.20	10.1/000
F ₁ -P ₂	+0.27	0.54	-2.6/000	42.1/000
Backcross (B ₂) Comandor × F ₁ (♀Excelsior × ♀Comandor)	18.46	1.06	10.66	55.94
B ₂ -P ₂ Comandor	+2.87/**	1.14/00	-1.34	+0.97
Mutants V ₂ Comandor	19.56	0.99	19.56	58.37
Standard Comandor V ₀	15.6	2.20	12.00	55
V ₂ -V ₀	3.97/***	-1.21	7.56/***	3.37/***
F ₁ Olimp × NJA19	19.56	1.10	8.74	69.50
F ₁ -P ₁ × P ₂	+5.38/***	-0.81	-0.79	6.07/000
F ₁ -P ₁	+3.0/***	0.10	-1.42	3.36/* **
F ₁ -P ₂	+7.76/***	-1.52	-0.16	15.5/000
F ₂ Olimp	17.78	1.06	12.25	62.26
F ₂ Olimp-P ₁ Olimp	+1.22	0.04	2.09/*	3.88/000

The 1:1 segregation of the fruit shapes pointed to the descendant 'Goldrich' acting as a double recessive tester, and confirmed the heterozygotic nature of the parent 'Excelsior' as illustrated by the mutual monohybridisations ♀ExcelsiorAa × ♂ComandorAa and ♀ComandorAa × ♂ExcelsiorAa.

Based on these results, the following conclusions can be summed up:

- Controlled hybridisation, backcross, and physical gene mutation were efficient methods to improve fruit quality.
- Transgressions of dry substance and vitamin C were revealed in apricot descendants F₁.
- There were positive non-linear genetic correlations between the content in soluble dry matter and vitamin C.
- The heredity of the fruit shape was dominant, with segregation 3:1 in the combination of ♀elongated fruit (Aa) × ♂elongated fruit (Aa), and segregation 1:1 in the combination of ♀elongated fruit (Aa) × ♂round fruit (aa).

BIOTECHNOLOGY OF APRICOT TREE IN ROMANIA

Micropropagation

Culture initiation

The reaction to micropropagation is genotype and explant type dependent, according to many research teams results (Butic-Keul *et al.* 2004; Corneanu *et al.* 2006; Pérez-Tornero *et al.* 1999; Pérez-Tornero *et al.* 2006; Rogalski *et al.* 2003).

In Romania, young shoots from the following cultivars were used for micropropagation: 'Best of Hungary', 'Favorit', 'Mamaia', 'Excelsior' and 'Comandor', hybrids: progeny F₂ 'Excelsior' × 'Comandor' and rootstocks: 'Stella' and 'Goldcot', from which apex, meristems, leaves and microcuttings were sampled (Table 5). The aseptic method of the biological material differed according to genotype and laboratory. The general scheme of sterilization was: washing in water, dipping into ethanol 70-95%, 10-60 sec; dipping into sterilizing agent (Domestos 20%, NaOCl 0.8-4%, HgCl₂ 0.3%), 10-20 min, three washes in sterile distilled water (Table 1). For culture initiation, MS (Murashige and Skoog 1962) and QL (Quoirin and Lepoivre 1977) media were tested. The culture mediums were supplied with different hormone balances and bioactive unconventional substances: DDW (30 ppm deuterium content) and magnetic fluids (Table 2). A multiple emulsion was used as a magnetic fluid. In multiple emulsions, the magnetic part was formed by iron oxides FeO and Fe₂O₃, in a ratio of 1:1 (magnetite), which was obtained by coprecipitation in an alkaline medium and stabilised with oleic acid (Minea *et al.* 2003). The medium was solidified with agar (7%), pH = 5.6-5.8. The culture vessels were maintained at 24-26°C, 16 h photoperiod, white light (Osram L58 w/30) at a PPFD of 35 μmol m⁻² s⁻¹.

The explants that gave the best results concerning viability, as well as future evolution, were the apices and apical meristems. Similar results were obtained by Pérez-Tornero *et al.* (2006) who also observed that the survival of the explants was higher when axillary shoots instead of meristems were used for the culture introduction. In the case of young leaves and microcuttings, the shoots viability was low (under 20%), and total necrosis of the explants was observed 20 days after culture initiation. Shoot development and the elongation process were dependent on the genotype, as well as on the phytohormone balance of the culture medium (Table 6).

In the cultivars 'Best of Hungary', 'Favorit' and 'Mamaia' (Butic-Keul *et al.* 2004), the best results were obtained on the MS medium + 14.9 μM/l 2 iP (N⁶-2-isopentenyl adenine) + 0.5 μM/l IBA + 0.26 μM/l GA₃ (gibberelic acid) + 56.8 μM/l vitamin C + 54.3 μM/l adenine sulphate, the length of the obtained shoots being 10.6-19.6 mm. In the rootstocks 'Stella' and 'Goldcot', better development was noticed on the basal QL medium supplied with 2.2-4.4 μM BA + 0.5 μM NAA + 0.02 μM GA₃, in comparison with the basal medium MS with the same phytohormone supplement, the regeneration percentage being 19.7-21.6% (Popa *et al.* 2005). By comparing the reaction to micropropagation of the cultivars 'Excelsior' and 'Comandor' with their hybrid progeny F₂, Corneanu *et al.* (2006) pointed out that the heterosis effect was also manifested *in vitro*, while the hybrid had the best results as far as the main shoot elongation and the basal caulogenesis process were concerned. The authors emphasised the specificity of the reaction, depending on the genotype × culture medium interaction, observation sustained also by Pérez-Tornero and Burgos (2000a) in their studies on the micropropagation of the cultivars 'Bulida', 'Helena', 'Canino', 'Lorna'. Corneanu *et al.* (2006) observed improved results when the culture medium was prepared with DDW, as the shoot elongation process and the foliar organogenesis were significantly increased.

According to Pérez-Tornero and Burgos (2000a), MS is the worst medium for some sensitive genotypes (e.g. all plants died within six weeks in the cultivar 'Bulida', while in 'Currot' and 'Helena' explants showed hyperhydricity symptoms, due to the excessive ammonium content of the MS medium) in comparison with QL or modified WPM media. In opposition, the cultivars tested in Romania: 'Best of Hungary', 'Favorit', 'Mamaia', 'Excelsior', 'Comandor' and their F₂ progeny reacted very well on the MS medium and no vitrification processes were observed.

Secondary basal shooting process was dependent on the genotype, as most authors emphasised (Butic-Keul *et al.* 2004; Corneanu *et al.* 2006). In some cultivars on the initiation medium mentioned above, 1-2 basal shoots ('Best of Hungary', 'Favorit', 'Mamaia') were obtained, in others ('Excelsior', 'Comandor') no caulogenesis took place while, in the hybrid F₂ ♀Excelsior × ♂Comandor, 3-6 basal shoots/explant were obtained on the medium prepared with

Table 5 Asepsization methods in *P. armeniaca*. (Mihaela Corneanu and Viorica Bălan)

Genotype	Biological Material	Asepsitization methods	Laboratory	Reference
H ₁	Immature seeds	Ethanol 95%	Research Station for Fruit-Tree Growing Baneasa	Bălan <i>et al.</i> 1999
H ₂				
H ₃				
H ₄				
H ₅				
H ₆				
'Best of Hungary'	Shoots	Ethanol 70% -10 sec.	University Babes-Bolyai	Butic-Keul <i>et al.</i> 2004
'Favorit'		Domestos 20% -15 min.	Cluj Napoca	
'Mamaia'				
'Stella'	Shoots	Ethanol 70% -60 sec.	Research Station for Fruit-Tree Growing Băneasa	Popa <i>et al.</i> 2005
'Goldcot'		NaOCl 0.8% -20 min		
'Excelsior'	Shoots	NaOCl 4.0% -10 min.	University of Craiova	Corneanu <i>et al.</i> 2006
'Comandor'		HgCl ₂ 0.3% 10 min.		
♀Excelsior x ♂Comandor				

Table 6 Results obtained in *in vitro* culture initiation in *Prunus armeniaca* (cultivars, hybrids) in Romania. (Mihaela Corneanu)

Genotype	Explant	Optimum culture medium	Results	References
H ₁	IM	H basal; MS modified; LP modified	G	Bălan <i>et al.</i> 1999
H ₂				
H ₃				
H ₄		LP modified		
H ₅				
H ₆				
'Best of Hungary'	A	MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA ₃ + 10 mg/l vitamine C + 10.0 mg/l adenine sulphate	C, E, R	Butic-Keul <i>et al.</i> 2004
'Favorit'		MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA ₃ + 10 mg/l vitamin C + 10.0 mg/l adenine sulphate	C, E, R	
'Mamaia'		MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA ₃ + 10 mg/l vitamine C + 10.0 mg/l adenine sulphate	C, E	
'Stella'	AM	MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA ₃ + 10 mg/l vitamine C	R	
'Goldcot'		LP + 2.2 – 4.4 μM/l BAP + 0.5 μM/l NAA + 0.02 μM/l GA ₃	C	Popa <i>et al.</i> 2005
'Excelsior'	AM	MS + 1.0 mg/l BAP + 0.1 mg/l NAA	C, E	Corneanu <i>et al.</i> 2006
'Comandor'		MS + 2.0 mg/l BAP + 2.0 mg/l IBA + 0.1 mg/l GA ₃		
♀Excelsior x ♂Comandor		MS + 1.0 mg/l BAP + 0.1 mg/l NAA (prepared with DDW)		

Explant type: IM – immature embryo; A- apex; AM – apical meristem

Culture medium: MS modified; LP modified: ½ macroelements and iron; double microelements

Results: G – embryo germination; C – caulogenesis; E – shoot elongation; R – rhizogenesis

Table 7 The effect of the culture medium, deuterium-depleted water (DDW) and explant size on the main biometrical characters in hybrid progeny F₂ ♀Excelsior x ♂Comandor (Fisher's Test) (Corneanu *et al.* 2006).

Character	Fisher's Test					
	1- subculture medium; 2- DDW; 3- explant size					
	1	2	3	1 x 2	2 x 3	1 x 2 x 3
Shoot length	22.90***	0.60	356.97***	21.85***	6.89*	8.29***
No of leaves/explant	1.93	7.38**	87.34***	3.75**	5.03*	3.12**
Secondary shoot number	2.34*	21.51***	20.34***	1.52	5.13*	2.55*

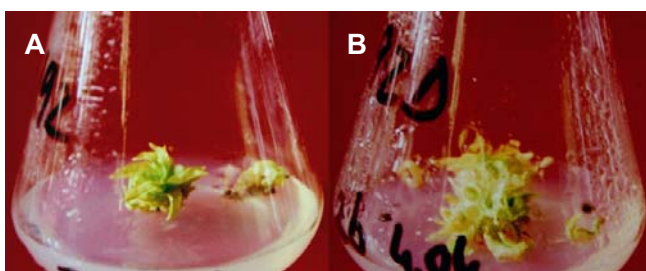
Table 8 Results obtained in *in vitro* subculture in *Prunus armeniaca* (inter- and intraspecific hybrids) in Romania. (Mihaela Corneanu)

Genotype	Explant	Optimum culture medium*	Results	References
H ₁	Germinated embryos	H basal; MS modified; LP modified	P; LS	Bălan <i>et al.</i> 1999
H ₂		H basal; MS modified; LP modified	P; LS	
H ₃		H basal; MS modified; LP modified	P; LS	
H ₄		MS modified; LP modified	P	
H ₅		MS modified; LP modified	P	
H ₆		LP modified	P	
♀Excelsior x ♂Comandor	Neoformed shoots 1 mm	MS + 2.0 mg/l BAP + 2.0 mg/l IBA + 0.1 mg/l GA ₃	E, CCE	Corneanu <i>et al.</i> 2006
(F ₂)	Neoformed shoots 3 mm	MS + 0.1 mg/l BAP + 0.1 mg/l IBA + 0.1 mg/l GA ₃ + 60 mg/l FM	E, R	

* Culture medium with best results

MS modified; LP modified: ½ macroelements and iron; double microelements

Results: R – rhizogenesis; P – plantlets; LS – lateral shoots; E – shoot elongation; CCE – caulogenesis via embryogenic calli; C – caulogenesis

**Fig. 5** The effect of DDW on caulogenesis process on *P. armeniaca*, F₂ hybrid ♀'Excelsior' × ♂'Comandor'. (A) MS + 1 mg/l BAP + 0.1 mg/l NAA. (B) MS + 1 mg/l BAP + 0.1 mg/l NAA prepared with DDW.

DDW (**Fig. 5**). DDW exercised an action of protection, both at the DNA and telomere level, which assured the chromosome integrity. But the telomere was partly lost with each cell division, therefore the division number characteristic to a cell was different (Hayfick, cited by Goodall 2003), depending on the genotype and probably on the tissue. The increase in the cell multiplication rate was limited by the telomere integrity. When the value (the division number characteristic to the genotype) was surpassed, cell divisions

became abnormal, either resulting in aneuploid cells or cell division stopped. The knowledge of this concept, as well as the use of DDW in culture medium preparation, can be a way of improving the *in vitro* multiplication rate of the explants.

Subculture

The neoformed shoots must be transferred (subculture) on a fresh medium after 30-45 days; after this period, apex necrosis occurs at a high rate, a fact noticed by other authors in the *Prunus* species (Scaltsioyannis *et al.* 1998); Balla and Vértesy 1999; Pérez-Tornero *et al.* 2000a).

In the experiments performed by Corneanu *et al.* (2006), the *in vitro* neoformed shoots of different size (3 mm, 4 leaves; 1 mm, 2 leaves) were transferred on eight variants of MS basal medium supplied with 0.44-8.9 μM BA + 0.5-9.8 μM IBA + 0.3 μM GA₃. The analysis of the effect of the subculture medium, initiation medium and explant type, pointed out the significance of each factor, as well as their interaction (Fisher's test) on the growth, differentiation and dedifferentiation processes. The main shoot elongation was very significantly influenced by the phytohormone balance, as well as by the explant size, a fact proved both by the Fisher's test and a very significantly positive correlation

between the shoot length and IBA quantity ($r = +0.3374$). The best results (shoot length = 20.5 ± 0.2 mm, 45 days) were obtained on the MS medium + $8.9 \mu\text{M}$ BA + $9.8 \mu\text{M}$ IBA + $0.3 \mu\text{M}$ GA₃. Organogenesis in the leaves was significantly stimulated in the medium variants where the auxin: cytokinin ratio favoured the latter. Caulogenesis at the shoot base was significantly influenced both by the phytohormone balance and the presence of DDW in the initiation culture medium, as well as by the explant size (Table 7). The highest number of shoots/explant was recorded in small shoots (1 mm) (8-12 shoots/explant), on the culture medium MS $8.9 \mu\text{M}$ BA + $9.8 \mu\text{M}$ IBA + $0.3 \mu\text{M}$ GA₃, originating from a medium prepared with DDW. This finding proves that DDW has a long term effect, having an important role in tissue rejuvenation. Knowing the effect of different controllable factors on the explant development allows their modulation in order to obtain the best results (Table 8).

Rhizogenesis

Obtaining strong rooted plantlets is a condition to carry out a successful acclimatisation process. Most authors observed that a reduced concentration to 2/3 -1/2 basal medium salts improved the rhizogenesis process (Balla and Vértsey 1999; Kamali *et al.* 2006). Some authors recommended an induction period in the darkness for certain genotypes (Pérez-Tornero *et al.* 2000a, 2006); however, this might produce chlorosis and shoot necrosis, fact confirmed by Koubouris and Vasiliakakis (2006) in the cultivar 'Babecou'.

In some genotypes, the rhizogenesis process can be obtained even in the initiation culture. Butic-Keul *et al.* (2004) obtained rooted plantlets on the same medium used for culture initiation, but the reaction to the culture medium was genotype-specific. The highest number of roots was obtained in the cultivars 'Best of Hungary' and 'Favorit' on MS + $4.9 \mu\text{M}$ 2 iP + $0.5 \mu\text{M}$ IBA + $0.26 \mu\text{M}$ GA₃ + $56.8 \mu\text{M}$ vitamin C + $54.3 \mu\text{M}$ adenine sulphate, while the cultivar 'Mamaia' rooted better in the absence of adenine sulphate. The number of neofomed roots in all the three genotypes was low (1.1-1.6 roots/explant) due to the low auxin content, as the culture medium was originally conceived to promote caulogenesis. Rhizogenesis occurred as a secondary process.

In order to induce rhizogenesis in the hybrid progeny F₂ ♀Excelsior × ♂Comandor, Corneanu (2006) supplemented the culture medium (80% MS) with phytohormones ($0.44 \mu\text{M}$ BA + $0.5 \mu\text{M}$ IBA + $0.3 \mu\text{M}$ GA₃) and different concentrations of magnetic fluid (0-200 mg/l MF). Roots were obtained on all the medium variants supplied with magnetic fluid, but in a concentration of 60 mg/l the number of roots/explant was maximum (4.6 ± 0.2), while shoot elongation showed a correspondingly positive reaction (20-24 mm growth of the transferred shoots in 45 days). In the control variant (non-MF medium), no rooting process was observed and the shoots length presented a significant negative difference in comparison with the variant MF (7-10 mm). No caulo- or callogenesis at the shoot base was recorded in any experimental variant. These findings are in accordance with the previous ones obtained by Corneanu *et al.* in other species: *Aloe arborescens* (1994), *Fragaria × annanasa* (1995), *Aztekium riitteri* (1996a), *Mammillaria duwei* (1996b), *Drosera rotundifolia* (1998), *Coryphantha elephantidens* (2000), *Prunus avium* (2004a), *Robinia pseudoacacia* (2004b) as a result of culture medium supplementation with magnetic fluid.

Immature embryo culture and seedling acclimatisation

The *in vitro* culture of immature embryos is an efficient method of rescuing the embryos resulting from interspecific hybridisation, as they frequently abort due to the genetic incompatibility between the embryo and endosperm.

In order to assure viable progeny of some interspecific hybrids, namely *P. armeniaca* × *P. persicum* (H₃) and *P.*

persicum × *P. armeniaca* (H₁, H₂, H₄, H₅ and H₆), Bălan *et al.* (1999) used culture immature embryos *in vitro* and obtained acclimatized plantlets (Table 8).

To initiate the *in vitro* embryo culture, immature embryos (1-10 mm) were used as explants, harvested 6-7 weeks after pollination, interspecific hybridation progenies ♀*P. armeniaca* (*P.a*) × ♂*P. persica* (*P.p*), ♀*P.p* × ♂*P.a* (Bălan *et al.* 1999): H₁ - ♀*P.p* × ♂*P.a* (♀Garden Delight × ♂B19/20); H₂ - ♀*P.p* × ♂*P.a* (♀Prios Magdalena × ♀Dacia); H₃ - ♀*P.a* × ♂*P.p* (♀Olimp × ♂PI 132030); H₄ - ♀*P.p* × ♂*P.a* (♀Michigan × ♂B4/73); H₅ - ♀*P.p* × ♂*P.a* (♀Sweet Gold × ♂Favorit); H₆ - *P.p* × ♂*P.a* (♀Platycarpa × ♂Dacia). To rescue immature embryos, three variants of hormone-free culture media were tested: H basal (Theobald and Hough 1960), MS modified and QL modified (double microelement quantity, half macroelements and iron quantities). In the embryo culture, the test tubes were maintained at 16°C in the darkness, until germination started, then transferred to 22-25°C with 16 h photoperiod.

As with neofomed shoot subculture, the explant size was as important as the culture medium composition. For the successful germination of embryos used as explants, they should be >4 mm in size, otherwise an *in ovulo* culture for 1-2 weeks is recommended before excision. This observation is in accordance with Burgos and Ledbetter (1993) who found that embryos between 5 and 9 mm germinated and developed into plants in a significantly higher percentage than the ones in more mature stages.

The hybrids H₁, H₂, H₃ reacted favourably in all the three variants of the culture mediums, resulting in 100% germination, while the hybrids H₄, H₅, H₆ recorded modest results (16-18%) on LP medium; H and MS mediums were inadequate, inducing germination inhibition. The mediums used for culture initiation were not always the best for plantlet development. A reduction to half the macroelements and iron, and the doubling of microelements stimulated embryo germination and plant development. The lower salts concentration improved rhizogenesis and subsequent plant growth after acclimatisation. The resulting plantlets were acclimatised in three steps: uncovering the test tube for 5 days (in the culture room), maintaining them for 10 days in the test tube with distilled water and then transferring them into pots and providing high humidity. 54% of the hybrid plantlets H₁, H₂, H₃ developed lateral shoots, with high number of leaves on short stems, proving the success of acclimatisation.

CONCLUDING REMARKS

In order to develop future breeding strategies, genetic engineering and the DNA technology may merge with other non-conventional methods, such as biotechnology (soma-clonal variation and stress resistance). For successful research, several things must be taken into consideration, among which increasing knowledge of the apricot genotype at the molecular level and the high expressions of inserted genes.

In the future, the studies based on molecular genetics and biotechnologies will be associated with biodiversity, and will employ combined research methods, such as: traditional hybridisation, backcross, mutagenesis, inbreeding, and unconventional genetic transformation. It is noteworthy that this strategy is already applied in some European countries: France, Italy, and Spain.

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Carmela variety and its author, Prof. Dr. Viorica Bălan