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Full Length Research Paper

Morphological and pomological traits of almond phenotypes (*Amygdalus communis* L.) isolated from their natural population

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Seven phenotypes were isolated from a natural population of almonds (*Amygdalus communis* L.) and more positive traits than the standard varieties that were cultivated, were identified. Over the period of three years, phenological and pomological research was conducted *in situ*, along with the observation of vegetative traits and productivity of isolated phenotypes. The research was conducted on the basis of the 2.11 (IBPGR) descriptor for the identification of *Prunus* varieties. Phenotypes K1, K4, K6 showed the best results. The research will be continued through a comparative experiment with the selected phenotypes in equal agro-ecological conditions.

Key words: Almond, selection, phenotypes, 2.11 IBPGR descriptor.

INTRODUCTION

In the narrow Mediterranean belt area of the Republic of Croatia, olive, wine grape, almond, fig, Maraska cherry and citrus fruits are cultivated. These crops represented the main source of income to local population throughout history.

Almond (*Amygdalus communis* L.) from Central Asia was spread all over the Mediterranean by the Phoenicians or Greeks (Godini, 2005). Nowadays, the production of almond is restricted to three world regions. These are Asian and Mediterranean countries and California, with limited amounts in Australia, South Africa, Chile and Argentina (Aslanta and Güleryüz, 2001).

Almond kernels are concentrated sources of energy with a significant share of fat, protein and fibers. Fats are primarily non-saturated, mostly oleinic and linoleic fatty acids. Non-saturated fatty acids are important in maintaining low cholesterol levels in the blood (Aslanta et al., 2001; Saura Calixto et al., 1981; Aslanta et al., 2001). Almond also contain significant amount of micro and macro nutrients (Aslanta et al., 2001). The kernel contains between 5.93 - 7.27% water, 8.03 - 8.13% ash, 53.67 - 54.26% oil, 23.03 - 23.98% proteins, 4.15 - 5.29% total sugars, 1546 - 1685 mg/100 g K, 253 - 259 mg/100 g P, 640 - 678 mg/100 g Ca, 447 - 494 mg/100 g Mg, 24.30 - 25.80 ppm Cu, 76.33 - 80.50 ppm Zn, 54.83 - 65.33 ppm Fe and 37.67 - 37.83 ppm Mn (Aslanta et al., 2001; Barbera et al., 1994).

Up to the 20th century, people in Croatia mostly bred almond cultivars for local consumption. As almond reproduced itself mostly generatively, the Croatian production potential is represented by a heterogeneous population, with irregular fertility, low production (averagely 6 kg/tree), low fruit quality, among others (Vlašić, 1979). In order to improve production and the quality of the locally bred almond cultivars, a project was initiated in 1990 to identify, gather and collect new almond phenotypes from the native populations (Mladar et al., 1990).

Therefore, the main aim was to isolate phenotypes that had more desirable traits, such as late inflorescence, a higher degree of autogamy, a smaller percentage of double kernels and a higher percentage of pollen grain germinability.

MATERIALS AND METHODS

On the entire Dalmatian cultivation area, 13 almond phenotypes were selected through a method of individual selection (Tavčar, 1959). The basic criterion for phenotype selection was their inflore-

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Table 1. Growing area of selected Almond phenotypes

Chosen Phenotype	Place of Research	County	Field type	Average tree age
K 1	Gustirna	Šibensko – kninska	Southern slopes	31
K 2	Dograde	Splitsko – dalmatinska	Field	42
K 3	Dograde	Splitsko – dalmatinska	Southern slopes	120
K 4	Seget Gornji	Splitsko – dalmatinska	Field	30
K 5	Smokvica	Dubrovačko – neretvanska	Southern slopes	170
K 6	Čara	Dubrovačko – neretvanska	Southern slopes	47
K 7	Čara	Dubrovačko – neretvanska	Field	37

Table 2. Full bloom date of chosen phenotypes and control cultivars.

Phenotype	K1	K2	K3	K4	K5	K6	K7	Non Pareil	Texas	Jaltinski
Full bloom date	24.03	13.02	17.02	10.03	16.02	06.03	16.02	26.02	04.03	08.03

scence period, that is, late inflorescence. The analysis of results obtained after a 4-year research led to the isolation of 7 phenotypes with more positive traits than the standard varieties that were currently cultivated. Cultivation areas of the 7 selected phenotypes are presented in Table 1.

The research was continued *in situ* with the 7 selected phenoltypes and the results were for the past three years. The 2.11 descriptor (IBPGR) (International Bureau of Plant Genetic Resources) (Perret, 1988) for the identification of *Prunus* varieties, was used. Each phenotype was represented by one tree. For each phenotype, the date of its full inflorescence was recorded. The research of the success of autogamy and open pollination was conducted. The success of autogamy was determined by selecting and isolating fertile branches before the flowers opened. The aim of determining the percentage of open pollination before flowering resulted in the selection of four branches on each examined tree. These branches were covered with linen bags (North – East – West – South) and the number of flowers was counted. The counting of pollinated fruits was conducted 20 days after full bloom and it was expressed by the percentage in relation to the number of flowers present.

The germination of pollen was conducted *in vitro*. The branches with flowers that were still in their white capitula phase (balloon stage) were picked and brought into the laboratory. During pollination, the germinability of the pollen was determined by the pendant drop method in a 12% sucrose solution. After 12 h, using a Litz light microscope, the amount of germinated and non-germinated pollen grains were counted and are presented as percentage of germinability.

Tree exuberance was also determined. The surface of the leaf (cm^2) was measured with the Portable Area Meter model Li 3000 (Li-Cor).

The average value of leaf surface was determined on the basis of 100 leaves per sample. The measured productivity per tree was expressed in kilograms.

The pomometric research on the fruit, length and width of the shell and kernel (mm), as well as fruit and kernel mass (g) was conducted. The reproduction of the kernel (%) was calculated as fruit mass in relation to kernel mass. The indices of the shell and kernel shape were determined by the relation of width and length. The percentage of double kernels was also noted and the external appearance of the shell was determined by visual method. Taste of the kernel was determined by organoloeptic method and the softness of the shell was determined by the methods described in the 2.11 descriptor (IBPGR). Each sample contained a number of 100 fruits.

The phenotypes were sorted into particular groups on the basis of the examined traits, according to the IBPGR 2.11 descriptor. As control samples, the following varieties «Texas», «Non Pareil» and «Jaltinski» were used.

Research results were processed by variance analysis and the mean value differences were tested with a t-test at a significance threshold, p = 0.05.

RESULTS AND DISCUSSION

Phenological research

Flowering

A very important characteristic of almonds is their flowering period. Since almonds flower very early, at the end of winter or in early spring, this often results in damages by late frost as well as low pollination and fecundation during cold, cloudy and rainy weather (Vargas and Romero, 2001). Late flowering is the aim of most almond breeding programmes because of a smaller possibility of spring frost and temperatures that are more favorable for pollination and fecundation (Grasselly and Cossa-Raynaud, 1980; Vargas et al., 1984; Kester and Gradziel, 1996; Monastra and Raparella, 1997; Socias et al., 1999).

K2 was the earliest flowering phenotype (13 February) and K1 was the latest flowering phenotype (24 March) as shown in Table 2. According to Miljković (1991), «Texas» and «Jaltinski» varieties were rated in a group of late-blooming varieties, while "Non Pareil" was rated as a medium-late flowering variety. On the other hand, Vargas and Romero (2001) classified the "Non Pareil" variety as a mid-early flowering variety, while «Texas» as late and «Jaltinski» as a very late flowering variety.

According to the IBPGR descriptor 2.11, the examined K2 phenotype could be classified into the group of early flowering almond varieties, phenotypes K7, K5 and K3

Table 3. The percentage (%) of self-compatibility, open pollination, pollen germination and pollen grains normal development of chosen phenotypes

Phenotype	K1	K2	K3	K4	K5	K6	K7
Open pollination (%)	37.00	30.00	40.00	35.00	32.00	41.00	32.00
Self – compatibility (%)	26.33	21.13	18.02	22.17	18.00	21.83	21.00
Pollen germination (%)	61.90	67.20	69.80	76.30	67.30	69.60	73.00
Pollen grains normal development (%)	81.50	85.50	89.20	91.90	90.00	92.20	89.50

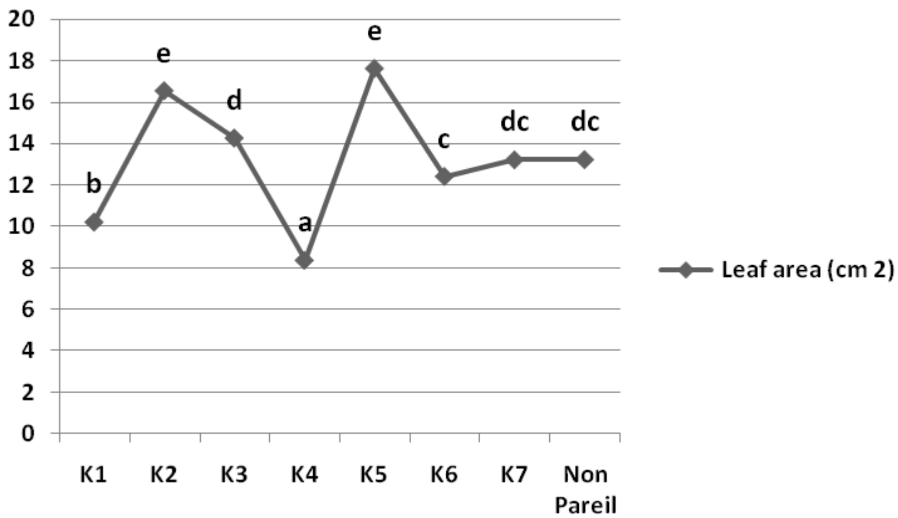

Figure 1. Leaf area (cm^2) of chosen phenotypes and control cultivar (significant differences are indicated by different letters (p = 0.05)).

could be classified as mid-early flowering varieties and phenotypes K6 and K4 as very late flowering varieties. Phenotype K1 could be classified as an extremely late flowering variety. According to the same descriptor, «Non Pareil» is a mid-late flowering variety and «Texas» is a late flowering variety.

Autogamy, open pollination, germinability

Most almond varieties were open-pollinated, thus it was necessary to use two or more mutually compatible varieties in plantations. The need for synchronous flowering of different varieties due to open pollination has its disadvantages. There are different factors that prevented successful cross pollination, such as insufficient overlapping of varieties in bloom, absence of bees, bad weather, among others (Dicenta et al., 2001). Furthermore, the early reception of pollen from an open-pollinated almond variety, may limit the space necessary for the reception of compatible pollen. Due to heterotrophic trait of the pollen tube growth, pistil reserves may be reduced (Egea et al., 2001; Herrero and Dickinson, 1979) and may prevent the development of the pollen tube of the compatible pollen, as well as cell fecundation (Egea et al., 2001; Herrero and Dickinson, 1981). Thus, the aim of many almond breeding programmes was to obtain open-pollinated varieties (Monastra et al., 1988; Legave et al., 1997) as a prerequisite for establishing mono-cultural plantations in order to avoid the above-mentioned problems.

All selected phenotypes have shown a satisfactory degree of autogamy (Table 3), due to no satisfactory statistical differences. The research showed good pollen germinability (between 61.9 and 76.3%) as well as a high percentage of normal pollen grains (81.5 to 92.2%) (Table 3).

Vegetative traits

Leaf surface and tree exuberance

The selected phenotypes have shown a great variability in the surface of the leaf and statistically significant difference was determined between them (Figure 1). Phenotype K5 had the largest leaf surface (17.6cm^2) while phenotype K4 had the smallest leaf surface (8.36cm^2). Palasciano et al. (2005) stated that cultivated almonds (*A. communis*) had a leaf surface larger than 10 cm^2 while the leaf surface of wild almonds (*Amygdalus*

Table 4. Chosen phenotypes vigor.

Phenotype	K1	K2	K3	K4	K5	K6	K7
Tree vigor	Weak	Strong	Strong	Intermediate	Strong	Intermediate	Strong

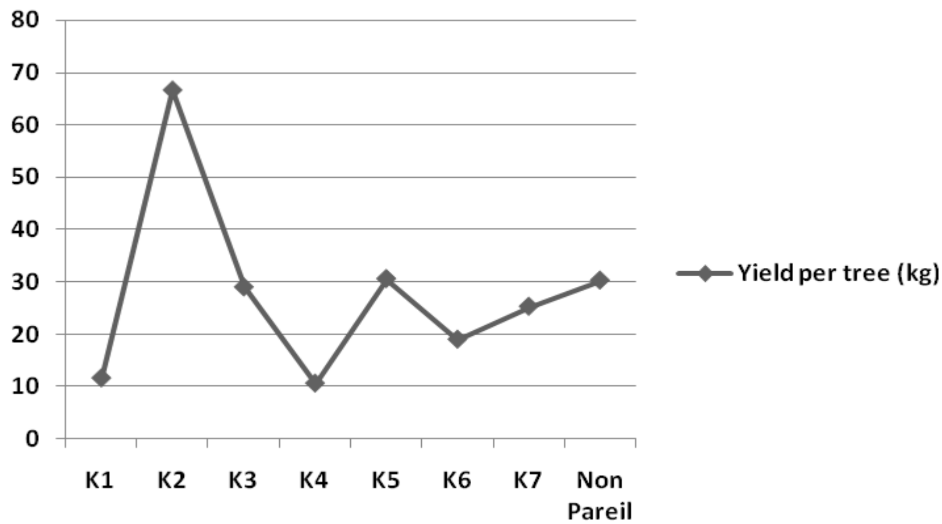

Figure 2. Yield per tree of chosen phenotypes and control cultivar (kg).

webbii Spach.) was less than 10 cm^2. According to same authors, out of all examined cultivated almond varieties, the smallest leaf surface of 13.1 cm^2 belonged to the «Marcona» variety. According to their research, the «Non Pareil» variety had a leaf surface of 13.4 cm^2 which matches the present research results regarding the same variety. Table 4 showed the exuberance of the selected almond phenotypes.

Crop

Production was determined as crop per tree (kg) for the selected phenotypes (Figure 2). Tree crop and exuberance were positively correlated, therefore the more exuberant the trees, the higher the crop. The highest crop belonged to phenotype K2 (66.67 kg/tree) and the lowest one to phenotype K4 (10.6 kg/tree). According to IBPGR descriptor 2.11, the «Non Pareil» variety was considered as a medium-productivity variety and based on this, it was concluded that phenotypes K1, K4, K6 may be regarded as low-productivity phenotypes, phenotypes K3, K5, K7 as medium-productivity phenotypes and phenotype K2 as high-productivity phenotype.

Pomological research

Fruit mass

Significant statistical differences for fruit mass differences between the selected phenotypes (Figure 3) were obtained.

Furthermore, a significant difference between the selected phenotypes and standard varieties «Texas» and «Non Pareil» was also determined. The IBPGR descriptor 2.11, states that the «Texas» variety has tiny fruits and the «Non Pareil» variety has medium-large fruits. According to the results of this project, fruit mass of «Texas» corresponded with the report of Gizdić (1997), Manušev et al. (1978), while Ak et al. (2005) reported an average value of 1.57 g and Vlašić (1976) 3.32 g. The average fruit mass for «Non Pareil» was 1.86 g (Ak et al. 2005). According to the IBPGR descriptor 2.11, phenotype K5 can be calssified into the group of varieties with tiny fruits, while phenotypes K6 and K7 belonged to the group with medium-large fruits. The remaining phenotypes belong to the group of larger fruits with phenotype K1 being the largest fruit (3.82 g).

Kernel reproduction

There was a significant difference between phenotypes as well as between phenotypes and standard varieties (Figure 4).

The most favorable kernel reproduction belonged to «Non Pareil» and phenotype K6, although no significant difference was obtained between them. «Non Pareil» and «Texas» had a lower kernel reproduction than the values obtained from literature (Ak et al., 2005; Caglar et al., 2005;

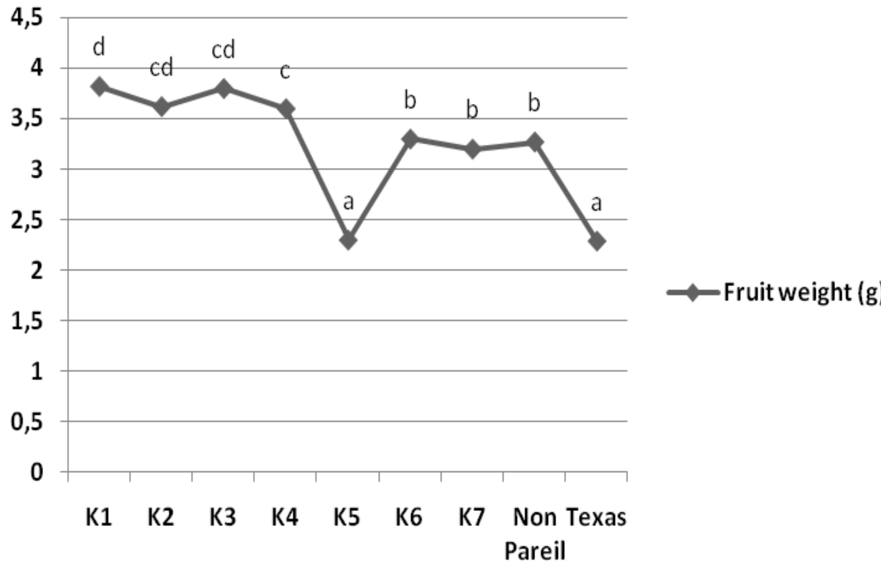

Figure 3. Fruit weight (g) of chosen phenotypes and control cultivars (significant differences are indicated by different letters (p = 0.05)).

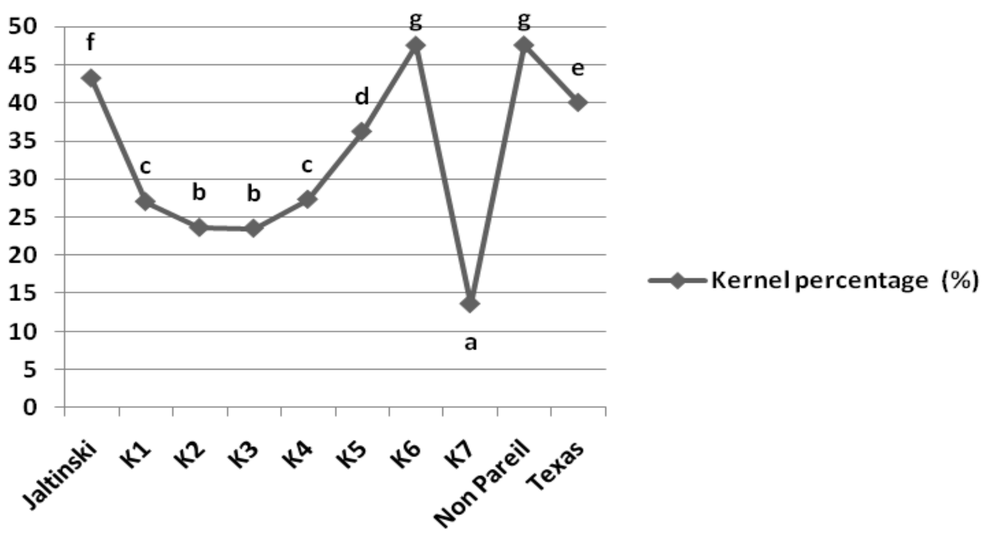

Figure 4. Kernel percentage (%) of chosen phenotypes and control cultivars (significant differences are indicated by different letters (p = 0.05)).

Table 5. Duble kernel rate (%) of chosen phenotypes and control cultivars.

Phenotype	K1	K2	K3	K4	K5	K6	K7	Non Pareil	Jaltinski
Double kernel (%)	13.20	0.30	0.70	10.60	1.60	2.50	0.20	27.00	24.67

Gizdić,1997). The kernel reproduction value for «Jaltinski» were somewhat lower as determined by Kaska et al. (1998) and Caglar et al. (2005) than the results from this project. The lowest kernel reproduction was for phenotype K7 (13.6%).

The influence of the environment on the occurrence of double kernels has been known (Kester and Asay, 1975; Spiegel-Roy, 1979). One of the reasons can be low temperatures during the flowering period (Spiegel-Roy and Kochaba, 1974). Table 5 presents the percentage of double kernels in the selected phenotypes. The lowest percentage of double kernels belonged to phenotype K7,

Table 6. Shell and kernel characteristics of chosen phenotype.

Phenotype	K1	K2	K3	K5	K5	K6	K7
Softness of shell	Soft	Intermediate	Intermediate	Intermediate	Paper	Soft	Hard
Marking of outer shell	Densely pored	Intermediate	Intermediate	Intermediate	Without pores	Intermediate	Intermediate
Kernel taste	Sweet	Sweet	Sweet	Intermediate	Intermediate	Intermediate	Sweet
Nutshape	1.26	1.26	1.18	1.16	1.63	1.63	1.45
Kernel shape	0.68	0.45	0.51	0.68	0.46	0.62	0.70

and the highest percentage was phenotype K1. The percentage of double kernels in control varieties was two times higher than in the selected phenotypes. Caglar et al. (2005) recorded that «Non Pareil» had no double kernels, while Ak et al. (2005) confirmed the results of this project.

Kernel and shell traits

A short review of shell and kernel traits is represented by Table 6. The softness of the shell of phenotypes K2, K3 and K4 corresponded with the softness of the shell of «Texas», while phenotype K5 had the softness of the shell equal to «Non Pareil». According to the 2.11 descriptor (IBPGR), «Texas» has a medium-soft shell, while «Non Pareil» has a paper-like shell. Phenotypes K1 and K6 had a small shell, that can be broken by hand, while hammer was needed for breaking the shell of phenotype K7. Gizdić (1997) classified the hardness of the shell and kernel reproduction into five different groups. However, the results from this project could not confirm these groups.

According to the descriptor 2.11 (IBPGR), the shape of the kernel in phenotypes K2 and K5 can be classified as narrow kernels (radius width/ length from 0.41 to 0.48 mm) and phenotype K3 is classified as medium-wide kernels (0.49 - 0.55 mm). A wide kernel (0.56-0.65 mm) is characteristic for phenotype K6, while phenotypes K1, K4 and K7 have an extremely wide kernel (> 0.65 mm).

The taste of the kernel is a sensoric trait that varies on the basis of the genotype or variety. It can be classified as sweet, medium-sweet or bitter cultivars. Bitterness of almond seeds after grinding or chewing led to the hydrolysis of amygdaline which enzymatically transforms itself into benzaldehyde and cyanide which was poisonous (Vargas et al., 2001; quot. Conn, 1980). A bitter taste is a serious defect and the belief of possible toxicity. In other words, bitterness is a chemical defect that originates from wild almonds (*A. webbii*) due to a natural protection mechanism (Vargas et al., 2001).

As presented in Table 5, phenotypes K1, K2, K3 and K7 showed a sweetish taste, organoleptically similar to «Non Pareil». The taste of phenotypes K4, K5 and K6 were more similar to «Texas», thus it could be classified as medium-sweet. The sweetish taste is the result of the low level or absence of glycoside amygdaline (Vargas et al., 2001).

Conclusion

The potential of almond production in Croatia are represented by one very heterogeneous population. According to our research, phenotypes K1, K4 and K6 have shown satisfactory results (flowering date, fruit mass, kernel reproduction, percentage of double kernels, kernel sweetness and level of autogamy), while the production was rather low.

The research will be continued through a comparative experiment with the selected phenotypes in equal agro-ecological conditions with the aim of improving production rates and creating prerequisites for further selection programmes in almond culture breeding.

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