Walnut breeding in order to release new late leafing and lateral bearing cultivar(s)
Summary report about the period between 1st September 2017 and 31st August 2021

WP1 Evaluation of a double selected Persian walnut population

Weather conditions under harvest of 2018 and 2019 years were ideal; there were no delays in the ripening time. In 2017, first half of September was dry, therefore the ripening time delayed some days (7 to 10 days compared to a normal year), but second half of the ninth month was wet, so the delays in ripening time had been decreased. In 2020, September was dry also, so there was delays in ripening time, especially the genotype with early ripening time opened their green husks 7 to 12 days later, than as usual.

28 genotypes produced first grade nuts (lower border of the first grade nuts is 32 mm in diameter). The genotypes called V/2/8-10, V/3/44, V/4/6, V/3/30-31 derived from Milotai 10 and Pedro cross, V/2/28-30, V/1/26-28, V/1/14-16 derived from Pedro and Alsószentiváni 117 cross, V/4/78, V/4/65-66, Érdió 1 (syn.: BD6) individual selected genotypes, V/4/36-38, V/1/18-20 from Alsószentiváni 117 x Pedro had the best kernel colour, kernel rate and cracking rate among them.

Spring of 2020 and 2021 were warm and very dry with some frosty mornings. However the genotypes survived the late spring frosts; we could check all characters, which were described in the proposal. During the summer periods of the project there was enough rain, which was good for the nut size and the kernel development.

In the year of 2019 we had problem with the walnut husk fly, which appeared in the orchard. In the years of 2020 and 2021 we used chemicals and some experimental methods to decrease the spreading of walnut husk fly. The best combination was, when we sprayed above the canopies to reach as much as possible flies. Due to our novel innovation we decreased the ratio of walnut husk fly in our experimental orchard to zero.

One of our Persian walnut genotypes called BD6 was announced for state approval to the National Food Chain Safety Office (furthermore NFCSO) in Budapest on 7th December 2018, (its registration number at NFCSO is 470757). The growers, who are interested in our activities, could test it under their own fruit site conditions after signing a testing agreement. During the whole project period 187 grafted tree (1,87 ha) were planted out with BD6 genotype in 12 fruit site countrywide.

WP2 Enzymatic background of the winter hardiness of Persian walnut

If walnut production is to be profitable, however, the growing site requirements must be fully met. Hungary is located on the Northern border of European walnut production areas. Walnut has poor ecological adaptability, as its cultivation is greatly influenced by low temperatures during the winter dormancy period and in early spring. The breeding activities conducted in various countries are therefore of great significance.

In Hungary the most critical weather events for walnuts are the frequent frosts in early spring. Buds therefore need to burst late to avoid damage to the flowers. The frost hardiness aspect of trees is permanently changing. For the first time in Hungary, artificial freezing tests were performed to determine the frost tolerance of some Hungarian bred cultivars.

The greatest frost tolerance was recorded for the cultivar 'Tiszacsécsi 83' during the years tested, while the most sensitive cultivar was the control genotype, 'Pedro'. The frost tolerance data for 'Alsószentiváni kései' were similar to those of 'Tiszacsécsi 83', while those of 'Milotai 10' were almost the same as those of 'Pedro'. Differences were observed between the hybrids, 'Milotai intenzív' being frost-sensitive, 'Milotai bőtermő' and 'Milotai kései' moderately frost-tolerant and 'Alsószentiváni kései' frost-tolerant. The flower buds were the most frost-tolerant in January. The weather in the experimental years was characterised by slightly lower minimum temperatures in the first week of October, after which the temperature was similar to the long-term mean. The values recorded in January were probably equivalent to the genetically programmed optimum frost tolerance levels, but this needs to be confirmed in further research. The next substantial cold spell occurred in mid-December, which was followed by the second stage of hardening in the case of mixed buds. There was a further sudden intensive drop in temperature in mid-January, but by this time the mixed buds of walnut have reached maximum hardening. By March the continuous rise in temperature led to higher LT_{50} values, but as the winter weather was similar at the experimental location in all three years, no significant year effect was detected.

Biological results

The polyphenol recorded in the shoots during the winter dormancy period also exhibited a rise both over time and parallel to the drop in the external temperature. In summary it can be said that the peroxidase enzyme activity in the leaves increased in accordance with the frost sensitivity of the genotypes both as the vegetation period proceeded and in response to the

cold stress caused by a decline in temperature. It could be seen that both the rise in the peroxidase enzyme activity in the leaves and the accumulation of polyphenolic compounds in the uppermost internode could be good indicators of the diverse frost tolerance of varieties and of the stress effects to which they are exposed.

WP3 Walnut decline

Walnut decline is a known phenomenon by Hungarian growers, but its etiology has not been researched in our country yet. Main symptoms of walnut decline are as follows: the canopy of trees year by year increasingly looses their leaves becoming transparent, as a consequence of twig necrosis. Twig necrosis usually appears at first on the top of trees, later it occurs on the lower canopy levels. Necrosis finally extends to the whole crown of trees, and finally the trees die. Necrosis of woody parts (shoots, twigs, branches) often is accompanied by discoloration of nuts, as they are turning into black because of different infections. To invstigate this phenomenon, phytopathological surveys were made in Persian walnut orchards in 21 locations: Érd, Sarród, Nágocs, Visz, Lengyeltóti, Nagyoroszi, Horpács, Lovasberény, Tiszacsécse, Tiszakóród, Milota, Szeged, Túrkeve, Algyő, Szeged, Nagycsepely, Tiszacsécse, Tiszakóród, Milota, Tiszabecs, Kocs. Twig, fruit and leaf samples were collected, isolates were made from diseased parts of tissues. After morphological analysis we have found some well-known species, e.g. *Melanconium juglandina*, *Phomopsis juglandina*, *Neofusicoccum* sp, *Trichoderma* sp, *Penicillum* sp, *Diplodia* sp, and two different fungal pathogens were identified inside the *Colletotrichum acutatum* species complex. Presence of *C. acutatum sensu lato* on walnut is a novum in Hungary, and it was isolated from fruits, twigs, leaves, buds and stalks. Isolates were identified by molecular methods, based on ITS, actin and calmodulin genes. Molecular analysis of isolates was accomplished, the most important ones were deposited to the GeneBank of NCBI Database. Each of the isolates got into our genebank for long-lasting storage. It turned out that these fungi are *Colletortichum fioriniae* and *Colletotrichum godetiae*, the presence of both species are novelty in Hungary, and the presence of *Colletortichum fioriniae* on walnut fruits is only published in France all over the word. First report was published about this species in Plant Disease, with title: First Report of Walnut Anthracnose Caused by *Colletotrichum fioriniae* on English (Persian) Walnut Fruits in Hungary (2019). First report was published about occurrence of *C. godetiae* on walnut in the same paper, with title: First Report of *Colletotrichum godetiae* Causing Anthracnose and Twig Blight on Persian Walnut in Hungary (2021). According to our investigations, facultative parasites, weak parasites, wound parasites, which

are usually necrotrophic fungi, play an important role in walnut decline process, as well as these two newly found fungal pathogen species.

WP4 Improvement of new walnut rootstocks

In the frame of the project a genotype called Köpcös was selected for rootstock. This genotype indicated medium-strong vigour for the grafted scions, and not producing suckers. The genom of Köpcös is similar to Alsószentiváni 117, we tried to find some phenotypical differences in its bud take time, blooming characteristics, ripening time, nut characteristics. The Alsószentiváni 117 started to budbreak some day earlier, than the Köpcös. Both genotypes have a protoandric blooming, so the male flowers opened earlier, than the females. The female flowers of both genotypes started to bloom at the same time, but the first male flowers of Alsószentiváni 117 started to bloom earlier compared to the Köpcös. The nut size of Köpcös is very small, appox. half than Alsószentiváni 117. The germination rate of Köpcös was approx. 80%, which is very high.

Due to excellent characteristics of Köpcös some virus-free trees were planted in our Nuclear Stock Garden in fall of 2019. We maintained this plantation during the remaining project period.

WP5 Dissemination

In the frame of the project there were published 22 papers in total, of which 6 were published in the pre-reviewed journals (cumulated IF of the papers published during the project period is 18,88), and other 7 papers were as abstracts at different symposia and conferences and 4 proceedings were printed in English. Furthermore, 1 paper was published as abstract, another one as proceeding as well as another one as in a pre-reviewed journal in Hungarian language during the project period. Two papers (in Hungarian and in English one of each) were made public in magazines for growers.

Furthermore, two papers were submitted to the pre-reviewed journals, which are currently under review:

1. Geza Bujdoso, Asghar Soelimani, Benjamin Illes, Darab Hassani: Base temperatures comparison for leafing date, pistillate flowers receptivity and pollen shedding in Persian walnut (submitted to Erwerbsobstbau on 27th December 2020.),

2. Krisztina Szügyi Bartha, Géza Bujdosó, Éva Stefanovits-Bányai: Biochemical methods for the characterisation of walnut genotypes with different levels of frost tolerance (submitted to Erwerbsobstbau on 19th April 2021.).

During the project the Hungarian partners organised 3 events (in Budapest on 8th May 2018., in Budapest on 8th November 2018., in Körmend on 6th November 2019.) called “Persian walnut Day” (“Dió Szakmai Nap”) for small groups of growers planning the walnut growing, where the results from the project disseminated partly.

One PhD student, was working on the WP2, made her closing PhD exam (on 6th May 2019) and her first PhD defence (on 29th January 2021) during the project period.

Before Covid-19, the Hungarian partners had visa problem, therefore it was not possible to visit the Iranian partner. Under the Covid-19 outbreak the Hungarian and Iranian partners had the same problem with exchange of the researchers during the 3rd and 4th project years, we could not visit each other.

In the frame of the project a novel bred walnut candidate called Érdió 1, was announced for state approval, was applied for the patent in the territory of the European Union under 20203132 reference number at Community Plant Variety Office. Now we are searching for the possible partners, who are planning to propagate it.