Xanthomonas resistance in Hungarian spice pepper varieties

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM Publishing House, Hungary

Márkus F.[1], Kapitány J.[1], Csilléry G.[2], and Szarka J.[3]

[1]Red Pepper Research-Development P.B.C., 6300 Kalocsa, Obermayer tér 9. Hungary
[2]Budakert Ltd. 1114 Budapest, Bartók B. u. 41. Hungary
[3]Primordium Ltd. 1222 Budapest, Fenyőpinty u. 7. Hungary

Key words: *Capsicum annuum*, spice pepper, pepper, *Xanthomonas campestris pv. vesicatoria*, resistance breeding, *Bs-2* and *gds* genes

Summary: With a view to further enhance the reputation of Hungarian spice pepper it was necessary to improve resistance to the bacterium *Xanthomonas campestris pv.vesicatoria*, the most dangerous pathogen of pepper varieties. From among the familiar resistance genes in Hungary only the gene *Bs-2* could provide sufficient protection against the aggressiveness spectrum of the bacterium species *X.c.pv. vesicatoria*. The first results of the resistance breeding are the spice pepper varieties *Kaldom* and *Kalorez*. In addition to the *Bs-2* gene attempts are also being made at building in a *gds* gene into pepper, a gene creating a general defense system, a different strategy towards *Xanthomonas campestris pv. vesicatoria*.

Introduction

Hungarian spice pepper breeding goes back to several century traditions. The continental climate of the Carpathian basin ensures special ecological endowments for Hungary, which create excellent conditions for spice pepper cultivation. The taste, flavor and aroma of the ground pepper made from the spice pepper grown here makes it a special Hungarian export item in addition to being an indispensable spice of the Hungarian cuisine. In the past years 8–10,000 t ground pepper has been produced on 6–7,000 hectares, of which 4–5,000 t is exported. Even today only Hungarian breeds are cultivated.

Breeding of spice pepper began in Kalocsa (Hungary) in 1917 for the first time in Europe. A significant turning point was in the selection breeding based exclusively on hot populations when in 1928, Ferenc Horváth selected stems without pungency from a hot population and they were the basis of spice pepper varieties without pungency at Kalocsa and Szeged *(Obermayer* et. al. 1938).

Beginning with the 1960s, the spice pepper varieties, which are the biological basis of cultivation today have been produced by cross-breeding *(Márkus* et. al. 1998, 1999, 2000, 2001a). These varieties have contributed to the technological modernization of cultivation and processing substantially.

Increasing requirements necessitated the production of varieties highly resistant to the most important pathogen of spice pepper, the *Xanthomonas campestris pv. vesicatoria* bacterium. The most modern biotechnological methods have been used in resistance breeding going on at the Kalocsa Research Institute since 1992 *(Mitykó* et. al. 1995, 1996). The first results of the resistance breeding are the spice pepper varieties **Kaldom** and **Kalorez** resistant to *X.c.p.v. vesicatoria* bacterium *(Márkus* et. al. 2001b).

Material and method

Resistance to the bacterium was tested on 3–4 leaf old plants cultivated in propagating boxes. The inoculate of 10^8 bacterium cell/ml concentration made from 24-hour culture of *X.c.pv. vesicatoria* bacterium was pressed into the leaves by syringe. Selection was carried out after 7-day incubation on the basis of tissue changes.

Observation

Spice pepper varieties must meet many different requirements. The quality and quantity of the raw produce in themselves are not sufficient since the produces have to meet several technological requirements. The ground pepper, which is the end product also has to meet strict storability and application requirements.

The climatic conditions defining the quality of the ground pepper, which is the end product and the conditions of the *Xanthomonas* bacterium epidemic are in close and sophisticated connection with each other. Abundant rain along with low temperature at the beginning of the breeding period in June is suitable for optimum development of plants but also for epidemics. If July, August and September are free from rain and there is a lot of sunshine, there will not be epidemic and neither the yield nor the quality will decrease. On the contrary if the temperature is low and there is a lot of rain in the given period both the yield and the quality will

Figure 1 Hyphae of *Sclerotinia sclerotiorum* packed into sclerotium.

Figure 2 Myceliums of mushroom packed into primordium

lead to 'water soaked' infiltrated spots. With the reduction of humidity these spots will disappear and the location of the spot will remain healthy, white. Later on crater-like pits appear at the place of the spots, because the tissue infected by *Pseudomonas fluorescens* and packed hard is not able to grow while the parts not infected will grow further *(Figure 3)*. Similar phenomena appear on tobacco (*Nicotiana tabacum cv. Xanthi nc.*) if the inoculum of *Pseudomonas fluorescens* or different *Xanthomonas* bacterium culture was injected by the method of Klement into very young leaf blade tissues bordered by veins. The affected tissues thickened, hardened and became chlorotic. Later on they did not grow as fast as the healthily growing leaf blade ribs bordered by neighboring, non-inoculated areas. Those ribs became swollen, which is a good indication of difference in growth *(Figure 4)* The test performed on very young leaves is suitable to detect only changes in inoculated tissues. The difference in growth in this case may lead to wrong conclusion, the reason of it is the significant growth of the very young leaf. Accurate information on the growth of the inoculated and non-inoculated tissues can be obtained only if an almost entirely developed old leaf with slow growth, or leaves not growing any more are challenged. To map the growth of tobacco leaves, a pathogen *Pseudomonas syringae pv. phaseolicola* producing exotoxin, a *Pseudomonas fluorescens*, a *Xanthomonas campestris pv. vesicatoria* and a saprophyte bacterium isolation are used.

As a result of inoculation with *P. s. pv. phaseolicola* bacterium, the tissues of almost entirely developed tobacco leaves lost their turgors in 24 hours and wilted. On the other hand the leaf blade bordered by veins and infiltrated by the other three bacterium isolates became strikingly swollen *(Figure 5)*. Later on the tissues destroyed by hypersensitive reaction dried, but the tissue with hump reaction showed only slight chlorosis *(Figure 6)*.

The infiltrated tissues of old tobacco leaves bordered by veins, which stopped growing, reacted with a tissue hump to inoculation with all the four bacteria. The tissue hump is

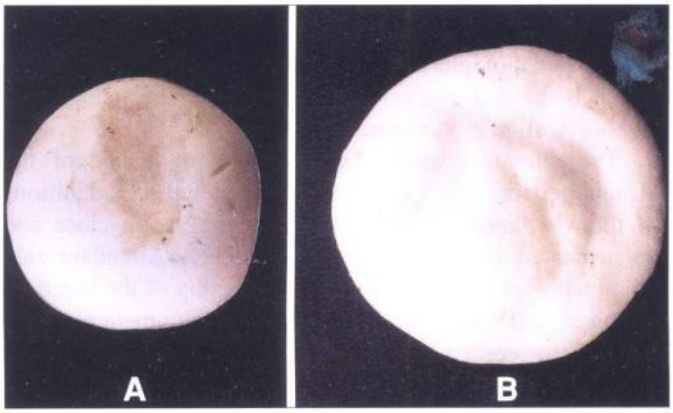

Figure 3 Water soaked spot (A) on mushroom pistil caused by *Pseudomonas fluorescens*, which is later overgrown by healthy tissue (B)

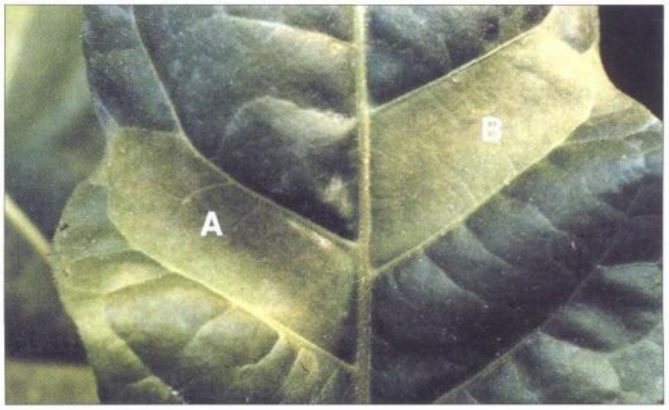

Figure 4 Tissue parts of very young tobacco leaf reacting by tissue packing to inoculation by bacteria *Pseudomonas fluorescens* (A) and *Xanthomonas campestris* pv. *vesicatoria* (B) later overgrown by healthy tissues.

possible only as a result of growth. Since the leaves of the plants may grow only with cell enlargement, we think it is proved that cell enlargement was caused by inoculation because this phenomenon is present even on old leaves. The extent of the hump of tissues shows the pressure among the cells pressed to each other as a result of cell enlargement. Cell enlargement is a defense mechanism with the purpose to eliminate the living space of microbes getting into the intercellular ducts.

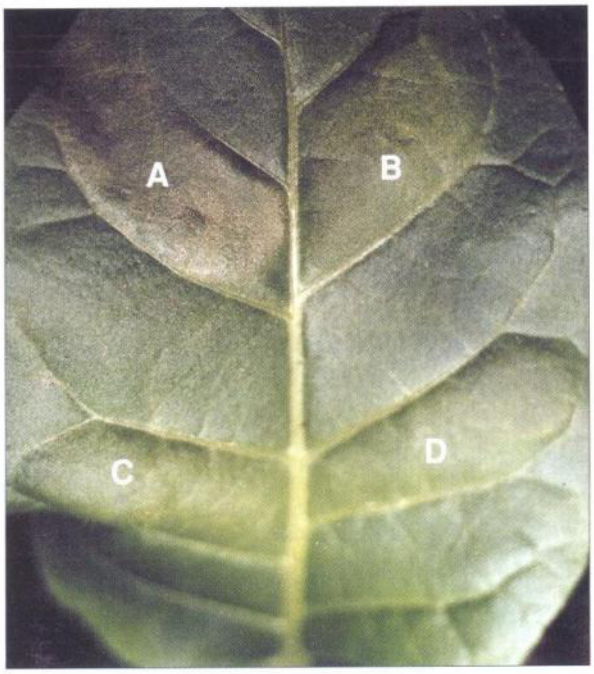

Figure 5 Developed tobacco leaf reacting to bacterium *Pseudomonas syringae* pv. *phaseolicola* (A) by turgor loss indicating the beginning of hypersensitive reaction while following inoculation by bacteria *Xanthomonas campestris* pv. *vesiatoria* (B), *Pseudomonas fluorescens* (C) and a saprophyte bacterium (D) it reacts by swelling indicating tissue packing

It is noteworthy that contrary to expectations, the *P. s. pv. phaseolicola* bacterium could not multiply in the old leaf with slower life processes to an extent, which could cause hypersensitive reaction. Consequently the tobacco also protected itself by tissue packing, which diminished the space of intercellular ducts.

In order to test the relation between hypersensitive tissue destruction resulting from unnatural overinfection by *Pseudomonas* bacterium species producing toxin, incompatible with tobacco and defense reaction based on tissue packing, the tissue of tobacco leaf was infiltrated in patches of different diameters. The tissues of infiltrated patches over 3 mm were destroyed by the hypersensitive reaction. The quantity of inoculum resulting in infiltrated patches less than 3 mm – which contained much more bacterium cells than can get into the leaf in the case of the contact of *Pseudomonas* bacterium species being in incompatible relationship with tobacco – could not lead to bacterium destruction. The infiltrated patches of less than 3 mm diameter showed only slight chlorosis proving that in the case of incompatible contact tobacco reacts with tissue packing. During the fast test elaborated by Klement to replace the hypersensitive tissue destruction and to determine bacterium species producing exotoxin, excessive number of bacterium cells were injected into the tissue of the tobacco leaf against which the defense system based on tissue packing has no effect under natural conditions.

The atypical defense reaction of plants against microbes caused by cell enlargement and based on tissue packing is observed on pepper, cucumber and beans *(Figure 7)*.

As a result of our breeding work the defense system based on tissue packing was improved so much that in pepper it provides defense even against its pathogen, the *Xanthomonas campestris pv. vesicatoria bacterium (Figure 7)*. The general feature of this defense reaction is proved by the fact that tissue packing also blocks saprophyte and pathogen microbes being incompatible with the pepper. Even the

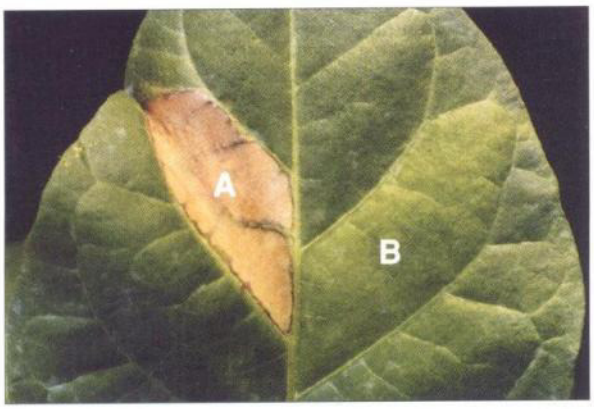

Figure 6 Hypersensitive tissue destruction induced by *Pseudomonas syringae* pv. *phaseolicola* (A) slightly chlorotic, swelling tissue packing caused by *Xanthomonas campestris* pv. *vesicatoria* (B) on tobacco leaf

Pseudomonas bacterium species producing toxin are unable to cause hypersensitive tissue destruction on it.

In addition to the resistance reaction of the cucumber, in the whole length of the leaf blade, against the *Pseudoperonospora cubensis* fungus based on hypersensitive tissue destruction, there was another reaction excluding the pathogen in the intercellular ducts by tissue packing. The infiltration of the pit of the abaxial surface, the disappearance of the intercellular ducts proves convincingly the increasing pressure between the cells with which the hump of the leaf surface keeps balance *(Figure 7)*.

The bean in addition to resistant symptoms based on hypersensitive reaction penetrating the whole thickness of the leaf blade against the pathogens of *the Pseudomonas syringae pv. phaseolicola* and the *Xanthomonas campestris pv. phaseoli* bacteria is able to hinder the invasion of pathogens also by tissue packing *(Figure 7)*. It is noteworthy that also in the case of cucumber, where the spore suspension was sprayed on the abaxial surface of the leaf, and beans, where the bacterium inoculum was spread by

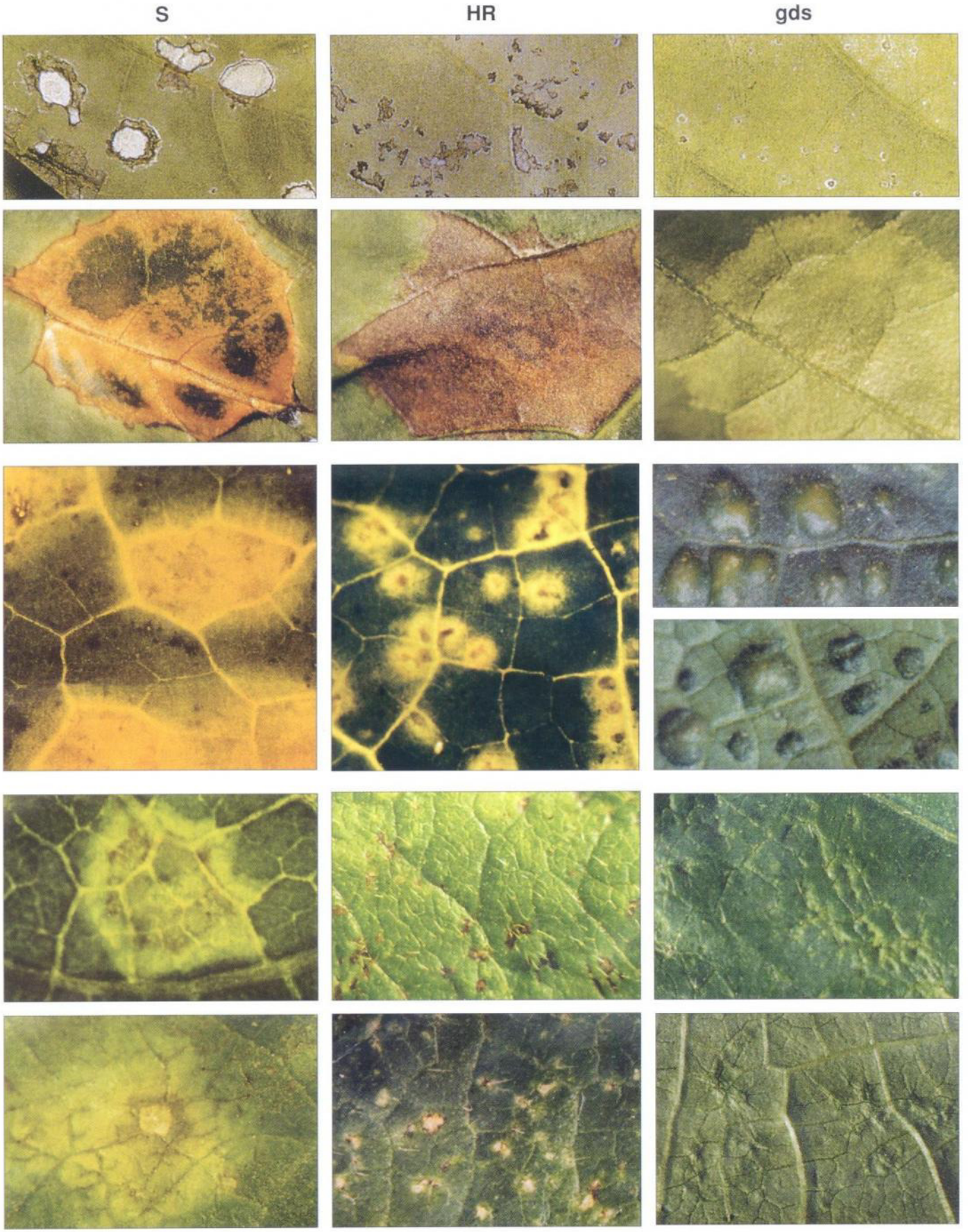

Figure 7 Symptoms indicating susceptible (S), hypersensitive (HR) and general defence system (gds) caused by bacteria *Xanthomonas campestris* pv. *vesicatoria* in pepper by *Pseudoperonospora cubensis* in cucumber and by *Pseudomonas syringae* pv. *phaseolicola*, *Xanthomonas campestris* pv. *phaseoli* in bean, respectively.

brush on the adaxial side of the leaf, hump referring to tissue packing developed from the opposite side, while on the inoculated surface pits or small craters appeared.

Conclusions

Plants in course of evolution and even today have been exposed to attacks of different microbes. If they reacted to all invasion attempts by hypersensitive tissue destruction they would lose a significant part of their leaf surface very quickly. That is why according to our assumption, in order to protect the assimilation surface, a different defense mechanism, which tries to preserve the cells and tissues developed and became common in the plant kingdom. This is suitable to defend the attacks of microbes being in incompatible relation with the given plant. Evolutionarily this plant reaction is probably older than the compatible host-pathogen relation between the host plant and its pathogens. According to our opinion resistance based on pathogen-specific hypersensitive tissue destruction developed only following the development of the host-pathogen relation as a defense reaction of the host plant.

Based on our tests the order of succession of the general defense system based on tissue packing is a recessive feature defined by a main gene *(Szarka & Csilléry* 2001), while hypersensitive resistance reactions are handed over by dominant gene/genes. In the case of compatible host-pathogen relation, the general defense of the host plant can be increased by accumulating the features strengthening and enriching the reaction, which provides perfect protection against the phylum of the given pathogen independent of its aggressiveness. In course of breeding a continuous series of the most efficient defense levels and transitions approximating susceptibility level are found. On the other hand in the case of resistance based on hypersensitive reactions, the symptoms enable only yes or no differentiation.

These genetic features also prove that tissue-packing reactions are very old. The observation that organisms of lower order (*Sclerotinia sp.*, mushrooms, *Agaricales*) can react to abiotic and biotic stress only with cell or hyphae packing also supports this assumption.

The basically two different processes of excluding pathogens based on tissue packing and hypersensitive tissue destruction, provide excellent opportunity to develop a new strategy of resistance breeding.

References

Holmes, F.O. (1929): Local lesions in tobacco mosaic. Botanical Gazette 87:39–55.

Holmes, F.O. (1938): Inheritance of resistance to tobacco mosaic disease in tobacco. Phytopathology 28: 553–61.

Klement Z., Farkas G. & Lovrekovich L. (1964): Hypersensitive reaction induced by phythopathogenic bacteria in tobacco leaf. Phytopathology 54: 474–477.

Stakman, E.C. (1915): Relation between Puccinia graminis and plants highly resistant to its attack. J. Agr. Res. 4: 139–200.

Szarka J. & Csilléry G. (1995): Defence system against Xanthomonas campestris pv. vesicatoria. Eucarpia IXth Meeting on Genetics and Breeding on Capsicum and Eggplant. Budapest, Hungary, August 21–25. 184–187.

Szarka J. & Csilléry G. (2001): General defense in the plant kingdom. Int. Jour of Hort. Sci. Budapest, 7. (1): 79–84.