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New Challenges in Hungarian Spice Pepper (Capsicum annuum L.) Improvement and Cultivation

Spice pepper in Hungary

In Hungary, spice pepper is in a special situation. growing spice pepper isa tradition in the southern part of Hungary and in this region many families and medium-sized farms depend on spice pepper production. Spice pepper powder is an essential part of Hungarian cuisine, for example in, Hungarian goulash, fish soup or other traditional dishes. Furthermore, the effect of spice pepper can be found in the folk art, for instance in the Kalocsai motifs. Spice pepper red powder is as close to the national identity of Hungarians as white bread or red and white wines, for example, Egri bikavér, Tokaji aszú *(Fig. 1)*. At last, this crop plant belongs to the Hungarian life, symbols, in short, to the Hungarian feeling.

Figure 1. *Spice pepper in gastronomy and folk art in Hungary*

Problems and challenges

In the last years, the economic situation of spice pepper has deteriorated in Hungary. A lot of farmers have given up growing this crop plant, so the growing area decreased, while the amount of imports (from Spain, China, South-Africa etc.) increased in the Hungarian markets. Some traders mixed the red pepper powder products derived from different countries. In this situation, the purchase of Hungarian spice pepper powder (origin: 100% Hungarian is almost impossible in the market. Furthermore, the problem in connection with toxin content (aflatoxin) of products muddied the reputation of Hungarian spice pepper.

In this case, each participant (researchers, breeders, farmers and traders) of the sector needs to find the opportunities to eliminate the risks of spice pepper-growing, production of powder and improve the competitiveness of the sector. The researchers and breeders have to find new genotypes and/or technologies which can improve the quality and quantity of the products, and the other participants of the sector have to utilize the advantages of the inventions.

Technology and breeding

In the case of some vegetable plants and flowers, the application of plant growing in greenhouse or plastic tunnel has improved the quantity and quality of the end-use product, safety of yield and the profit of farmers. However, these technologies require special genotypes with excellent genetic potential (yield, yield safety and quality). Such hybrids are preferred by the breeders which are able to fulfil these requirements.

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In case of spice pepper, one of the most important aims was to release hybrids for growing in plastic tunnels, which can give an alternative technology to the farmers parallel with the growing of spice pepper varieties at the field. The critical step in hybrid breeding is the selection and combination of the best parents to utilize the effect of heterosis. Following this assiduous work (more than 10 years), the basic genetic background (Hungarian and Spanish genotypes) was available for the test of the first hybrids. Parallel with these tests, doubled haploid (DH) plant production was started from the selected genotypes to get uniform hybrids.

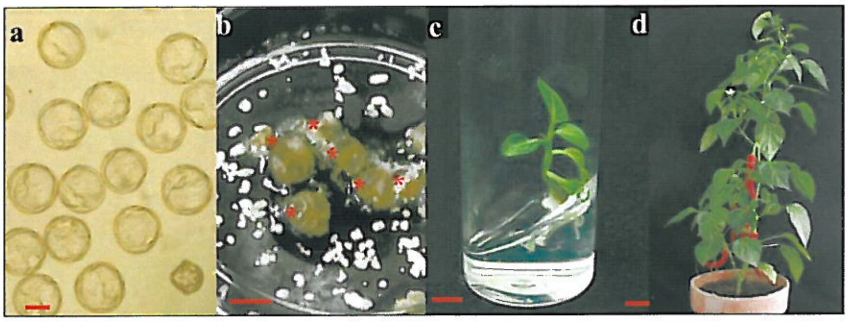

Figure 2. Characteristic steps of isolated microspore culture: a, freshly isolated microspores in optimal developmental stages (uninucleated and binucleated), b, five week old microspore derived embryos co-cultured with wheat ovaries (*), c, regenerated plantlet in glass tube and d, an acclimatized plant with flowers and fruits. Bars = 10 ìm for photograph (a), 1 mm for photograph (b), 5 mm for photograph (c) and 50 mm for photograph (d)

DH plant production as a tool in breeding

In the conventional breeding programs, at least 6-8 generations are necessary to breed new genotypes (varieties). DH plant production methods offer tools to produce quickly (1 generation) uniform homozygous lines for breeders, which can be integrated immediately into the breeding programme. Accordingly, the period of breeding can be reduced, and the DH plants and their hybrids will be uniform apparently.

Throughout the world, mainly in developed countries, breeders are interested in DH plant production methods, which can make the breeding more profitable. The application of DH lines is important in genetic studies since the use of uniform lines can furnish essential information on the genetic background of qualitative and quantitative traits associated.

In pepper, anther culture is one of the best-known methods for the production of doubled-haploid (DH) lines. The first reports of this method were published in the 1970s. In the past 40 years, this method has been subsequently modified and developed by different laboratories and applied in breeding programmes. Hungarian researchers focused also on the application of this method in their breeding and research programs (Mitykó et al. 1995, Gyulai et al. 2000, Gémes et al. 2009, Mitykó and Gémes 2006).

Despite the promising efforts, there are several factors (low efficiency and genotype dependence), which limited the application of it. Recently, these facts have initialised the researchers to develop new methods in DH plant production of pepper like *in vivo* polyembryony (Jedrzejczyk and Nowaczyk 2009), shed microspore culture (Supena et al. 2006) and isolated microspore culture (Supena et al. 2006, Kim et al. 2008).

The technique of isolated microspore culture has some advantages in comparison with other techniques: (1) microspores without somatic tissues are cultured in a liquid medium, so the developed embryos and regenerated plants derive surely from haploid cells and tissues, (2) the monitoring of the embryogenesis process is easier than in anther culture and (3) direct observation help the rapid optimization of culture conditions. Supena et al. (2006) discussed at first the microspore culture-derived haploids in Indonesian hot pepper. Later, Kim et al. (2008) reported on establishing haploids from the hot pepper 'Milyang-jare' using microspore culture, and optimized plating density of microspores as well as source and amount of added carbon in the medium.

In our experiments, optimal developmental stages (uninucleated and binucleated microspores) of isolated microspores were established *(Fig 2. a)*, the efficiency of culture (embryo production and plant regeneration) was improved by using ovary co-culture *(Fig 2. b)*. Green plantlet can be regenerated *in vitro* from the microspore derived embryos *(Fig 2. c)* which are able to acclimatize to greenhouse conditions *(Fig 2. d)*. The developed method was tested on various genotypes, and the effect of the genotype was obtained (Lantos et al. 2009). Further experiments are required to improve efficiency of the culture conditions.

The ploidy of the regenerated plants was checked by flow citometry *(Fig. 3)*, and the haploid plants were colchicine treated. The

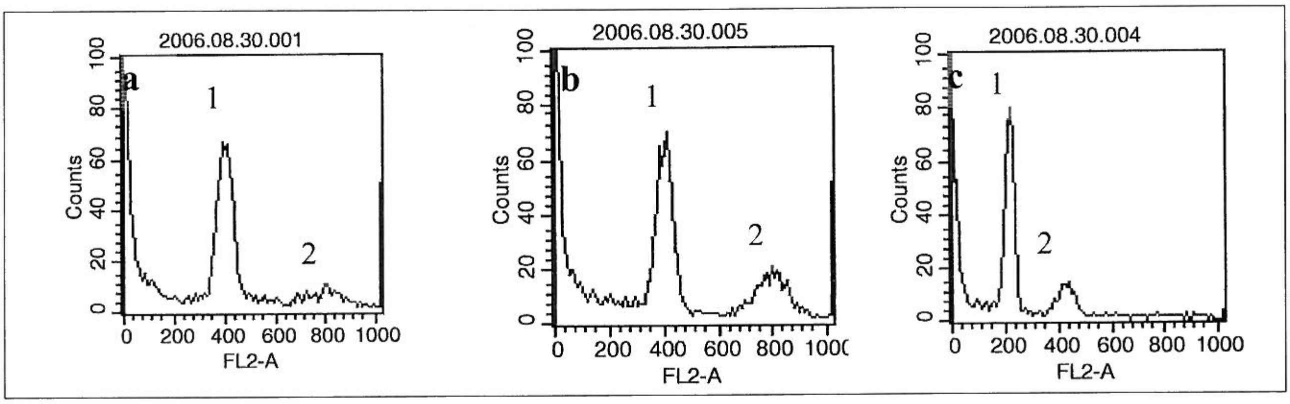

Figure 3. *Flow-cytometry ploidy level analysis of spice pepper plantlets. a Sample of a seed-derived control pepper plantlet. b A microspore culture-derived spontaneous diploid plantlet. c A haploid regenerant from a microspore culture. Peaks: a 1-2C 2-4C, b 1-2C 2-4C, and c 1-1C 2-2C*

Figure 4. *'Délibáb' spice pepper hybrid in plastic tunnel at different farms: a., Cultivation by using carnation net system. b., "Pepper wall" attatched to the string hanging system. c., Matured fruits of 'Délibáb' hybrid for first harvest at Ist week of August. d. The next harvest level of 'Délibáb' hybrid later by 2–3 weeks than the previous one*

spontaneous doubled haploid plants and colchicine treated doubled haploid plants were integrated into Hungarian spice pepper breeding program used as parent lines for hybrid seed production.

Registered spice pepper hybrids and their growing in the Carpathian Basin

Three spice pepper hybrids were produced by conventional hybrid breeding combined with biotechnological (DH parental line production) methods. These hybrids were released and registered in Hungary (Sláger – 2008, Délibáb – 2010, Bolero – 2010).

The hybrids are suggested for growing in non-heated plastic tunnel with intensive technology *(Fig 4)*. Following the technological requirements, these hybrids are able to produce high yields (1–1.5 kg /plant) and high quality paprika powder(350–420 ASTA, highest values). The risk of weather (abiotic stress) is limited in plastic tunnel, and the biotic stresses (insects, fungus, bacteria and viruses) can be prevented by chemical protection. The harvest is fluent from the beginning of August till beginning of winter (chillpoint). Work pick can be reduced in the farms and mills.

The combination of the new spice pepper hybrids with non-heated plastic tunnel cultivation offers an effective alternative opportunity for farmers to produce spice pepper powder with excellent quality. From 2008 to 2010, the number of farmers (approximately 100 farmers) and the growing area of hybrids (approximately 9 ha in plastic tunnel) are increasing fluently. These hybrids are grown by farmers mainly in the south part of Hungary (Bács, Békés and Csongrád counties). However, some tests are carried out in Slovakia and Romania. Serbian farmers are interested in applying the hybrids and technology. Therefore, the growing area is increasing in the Carpathian Basin.

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