Molecular identification of phytoplasmas associated with three floricultural species in Hungary

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Gaillardia aristata, *Echinacea purpurea* (Asteraceae) and *Aquilegia coerulea* (Ranunculaceae) are popular perennial garden plants. In 2017 and 2018, over 5% of *G. aristata* ‘Arizona Apricot’, *E. purpurea* ‘Cheyenne Spirit’ and *A. coerulea* ‘Biedermeier’ plants were affected by stunting, virescence and phyllody in a commercial nursery in Hungary. These symptoms were present in 182 out of 3500 *Aquilegia*, in 28 out of 520 *Echinacea* and 470 out of 10080 *Gaillardia* plantlets, with 5.2%, 5.38% and 4.66% infection rates, respectively. Leaf samples were collected from fifteen symptomatic and five symptomless plants of each species. Total DNA was extracted by CTAB method and nested PCR assays were performed using primer pair P1/P7 followed by R16F2n/R16R2 (Lee et al. 1998 and references therein). The 1.2 kb 16S rDNA fragment was amplified from each symptomatic plant separately. Restriction fragment length polymorphism analysis with *AluI*, *HaeIII*, *HpaII*, *MseI* and *RsaI* enzymes yielded restriction profiles identical to each other and to those of reference strain AY1 (GenBank accession number AF322644) belonging to the 16SrI-B subgroup (Lee et al. 1998). The P1/P7-amplified products obtained from *G. aristata*, *E. purpurea* and *A. coerulea* were cloned,

sequenced and deposited in GenBank under accession numbers MK992773, MK992774 and MK992775, respectively. BLAST searches of GenBank yielded 99.8–100% sequence similarities to ‘*Candidatus Phytoplasma asteris*’ strain SAY (AF222063).

In subsequent experiments, the elongation factor Tu (*tuf*), ribosomal protein (*rp*) operon (*rpl22* and *rps3*) and the molecular chaperonin *groEL* genes were examined according to Lee et al. (2004) and Mitrović et al. (2011). The PCR products obtained with primer pairs fTufI/rTufI, rpF1/rpR1 and AYgroelF/AYgroelR were cloned and sequenced (MN526022–MN526024). Identical sequences were obtained from the three plant species, sharing 100% identity with AY phytoplasma strain IRap (AJ271316), Onion proliferation phytoplasma strain OnP2 (GU228514) and AY phytoplasma strain AVUT (AB599686). The three phytoplasma isolates were classified into subgroups tufI-B, rpl-B and groELI-III. To our knowledge this is the first identification of ‘*Ca. P. asteris*’ in *G. aristata*, *E. purpurea* and *A. coerulea* in Hungary.

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References

- Lee IM, Gundersen DE, Davis RE, Bartoszyk IM (1998) Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int J Syst Bacteriol* 48: 1153–1169. <https://doi.org/10.1099/00207713-48-4-1153>
- Lee IM, Gundersen-Rindal DE, Davis RE, Bottner KD, Marcone C, Seemüller E (2004) ‘*Candidatus* Phytoplasma asteris’, a novel phytoplasma taxon associated with aster yellows and related diseases. *Int J Syst Evol Microbiol* 54:1037–1048. <https://doi.org/10.1099/ijs.0.02843-0>
- Mitrović J, Kakizawa S, Duduk B, Oshima K, Namba S, Bertaccini A (2011) The *groEL* gene as an additional marker for finer differentiation of ‘*Candidatus* Phytoplasma asteris’-related strains. *Ann Appl Biol* 159:41–48. <https://doi.org/10.1111/j.1744-7348.2011.00472.x>

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