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PROTOPLAST-TO-TREE SYSTEMS FOR *Prunus cerasifera* AND *P. spinosa* AS  
A TOOL FOR PHYLOGENETIC STUDIES WITHIN THE Prunoideae, Rosaceae

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The most important source of fruit cultivars for prunes and european plums, *Prunus domestica* L. ( $2n=6x=48$ ), was suggested to have originated as a natural hybrid between the fruit and rootstock diploid *P. cerasifera* Ehr. ( $2n=2x=16$ ) and the ornamental diploid species *P. spinosa* L. ( $2n=4x=32$ ), followed by the spontaneous chromosome doubling of the hybrid to produce a hexaploid. However, both *P. domestica* and *P. spinosa* appear to carry the *P. cerasifera* genome, suggesting a more complex origin for *P. domestica*. Neither theory can be put to the test using conventional methods, as sexual crossing between *P. cerasifera* and *P. spinosa* only yields triploid progenies. The somatic hybridization of both these species may produce the desired hexaploid genotype, but the establishment of plant regeneration from the cultured protoplasts of either or both of these genotypes stands as a prerequisite to achieve such goal. This communication reports on strategies that permitted the successful isolation of large numbers of highly viable mesophyll protoplasts for both *P. cerasifera* and *P. spinosa* and their conversion to whole plants. Following culture, using a modified MS-based medium, supplemented with NAA and zeatin (for *P. cerasifera*) or with NAA, BAP and zeatin (for *P. spinosa*), protoplasts underwent sustained division to the microcallus stage. After two successive subcultures for further proliferation, callus portions were transferred to a range of media based on MS medium and supplemented with NAA (0.01-0.025 mg/l), BAP (1.0-2.0 mg/l), zeatin (0.5-1.0 mg/l) and double the concentration of group B vitamins as in the original MS formulation, with shoot buds being regenerated from the protoplast-derived callus of both genotypes. These buds were subsequently propagated and rooted in vitro, and the whole protoplast-derived plants thus obtained were transferred to soil and acclimatized. The development, now, of efficient protoplast-to-tree systems for *P. cerasifera* and *P. spinosa* will enable the use of somatic hybridization to examine the theories on the origin of *P. domestica*, and studies of phylogenesis in the family Rosaceae generally.