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LETTER TO THE EDITOR

An alternative and ecological source of microprojectils for biolistic DNA delivery into plant tissues

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RNA silencing, a sequence-specific degradation mechanism of RNA, is considered one of the most efficient natural plant defense mechanisms against plant virus accumulation (*1)*. Transgenic plants carrying genome parts of target viruses have been constructed and shown resistant to viral infection (*2*). Alternatively, transient expression from a heterologous viral vector may activate plant's RNA degradation system (*3*). Therefore, preparative low-pressure-spraying of nucleic acids has potential application in agriculture to protect crops from viral infections similar to cross-protection by a mild virus strain (*4*).

Recently, we have published a simple and inexpensive method for biolistic transfection of plants by infectious cDNA of plum pox virus (PPV) using common air guns (*5*). The efficiency of this method was comparable with that using commercial gene gun machines. Initially, the technique was optimized for tungsten M-10 microcarriers (Biorad). However, similar to gold and other heavy metals, these carriers are unappropriate for a large scale application in open field.

To find an alternative inexpensive and ecologically innocuous delivery holding material, we tested a set of cheap and nontoxic silica- or phosphate-based potential DNA carriers, namely washed diatomaceous earth (Sigma), ceramic hydroxyapatite (Biorad), carborundum powder (600 mesh) and Celite 512 (Johns-Manville). Our experimental model included the PPV infectious cDNA clone pIC-PPV (*6*) and *Nicotiana benthamiana* under identical DNA binding conditions as described (*5*). From all alternative carriers examined in our experiments, only Celite 512 was able to deliver the cDNA succesfully into *N. benthamiana* plants and induce their infection as observed by symptom appearance and immunoblot detection. Although the efficiency of Celite 512 was more then twice lower then for tungsten M-10 (39 % versus 87 % of bombarded plants were transfected, respectively), these results showed that not only inert metals may serve as microprojectils in particle bombardment techniques. Although the Celite-based biolistics is not expected to reach the effectivity of application of the uniform metal particles, it may be improved by optimizing the DNA binding conditions or by increasing the shooting energy. Use of environmental-friendly delivery material in combination with a high-throughput method of delivering nucleic acids into plant tissues opens the possibility to use dsRNA-based "immunization" as a novel non-transgenic approach for virus control.

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Abbreviation: PPV = plum pox virus

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