onto the second leaf of 10- to 14-d-old seedlings by 4–7 d postinoculation (pi). Systemic symptoms appear as vein clearing, first, in a sector of leaf 3 or 4 by about 10–12 d pi, and then covering the whole leaf of all subsequent leaves to emerge. Vein clearing develops into vein banding by about 20 d pi, and uniform leaf chlorosis by 30 d pi. Leaves also develop as distorted, stunted structures. Different isolates of CaMV cause a range of variations on this theme from mild to severe symptoms. However, other *Brassica* hosts (e.g., *B. oleraceae* variants) develop markedly less severe symptoms, although *Arabidopsis* is one of the more susceptible host species.

Of those caulimoviruses that have been purified, the method used has been essentially similar to that originally described for CaMV ***(15)***, and upon which the method described here is based. The main problem to overcome in purifying caulimoviruses from infected tissue is disruption of the inclusion bodies containing the virions. In fact, two types of inclusion body have been identified in CaMV-infected tissues. The electron-lucent inclusion bodies, with a matrix consisting of the protein product of CaMV gene II and involved in aphid acquisition ***(16)***, contain relatively few virions. In contrast, the electron-dense inclusion bodies, with a matrix consisting of the viral gene VI product, contain most of the cellular virions ***(17,18)***. The CaMV virion itself is fairly robust and, depending on the isolate, can, under certain circumstances, withstand the rigors of incubation in phenol without releasing its DNA ***(19)***. The virion isolation method described here was designed to liberate virions from inclusion bodies by incubation of plant extracts in urea ***(15)***. Solublization of cells and prevention of virus aggregation is enhanced by inclusion of the nonionic detergent Triton X-100, and polyphenoloxidase activity is minimized by the reducing agent sodium sulphite. Liberated virions are purified by differential centrifugation. Virion DNA is released from purified virus by digestion with proteinase K. In the following protocols, we have included our standard method of CaMV isolation, which is based on a longer procedure originally described by Hull et al. ***(15)***, and a quick method for isolating CaMV DNA from smaller amounts of tissue. A further rapid method of isolating high yields of CaMV DNA has been described by Gardner and Shepherd ***(20)***. We also include description of a two-dimensional (2D) gel electrophoresis method we have used to study complex DNA populations, which has allowed us to characterize replicative forms of caulimoviral DNA ***(21,22)***.

2. Materials

2.1. Virion Purification

1. Infectious inoculum: Sap made by grinding 1 cm^2 infected leaf in 1 mL sterile water; purified virus; purified virion DNA; cloned virion DNA in TE (10 m*M* Tris-HCl, pH 7.8, 1 m*M* EDTA).
2. Celite abrasive.