Actin filament bundles are the backbone of cytoplasmic strands that traverse the large central vacuole in plant cells. We have used optical tweezers, combined with confocal microscopy to produce cytoplasmic protrusions and simultaneously study the actin organization. Actin filaments enter tweezers-formed protrusions in a myosin-dependent way. Depolymerization of actin filaments increases, and inhibition of myosin motor activity decreases the ability to deform the cytoplasm. This shows that actin filaments structure the cytoplasm in plant cells and determine its elastic properties in a myosin-dependent way. We use mutants, combined with localization studies to study the role of the actin filament bundling protein villin in the organization and deformability of the actin cytoskeleton and the cytoplasm in plant cells.