Preparation of megabase-size DNA from plant nuclei
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Abstract
A novel technique has been developed for the preparation of high molecular weight (HMW) DNA from plant nuclei. This technique involves physical homogenization of plant tissues, nuclei isolation, embedding of the nuclei in low-melting-point agarose microbeads or plugs, and DNA purification in situ. This technique is simple, rapid, and economical, and the majority of the DNA prepared is over 5.7 Mb in size. The genomic DNA content of the HMW DNA prepared by this technique is enriched by at least threefold and the chloroplast DNA content is reduced by over twofold relative to that prepared from plant protoplasts by existing methods. The DNA is readily digestible with different restriction enzymes and partial digestions of the DNA could be reproducibly performed. This method has been successfully used for the preparation of HMW DNA from a wide range of plant taxa, including grasses, legumes, vegetables, and trees. These results demonstrate that the DNA prepared by this technique is suitable for plant genome analysis by pulsed-field gel electrophoresis and for the construction of yeast and bacterial artificial chromosomes.