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Towards Improved Cell Cycle Synchronization and Chromosome Preparation Methods in Cotton

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Cotton is an economically important crop that needs more extensive genetic characterization. Highly effective methods for consistent cell cycle manipulation are needed to efficiently produce high quality cytological preparations of chromosomes, and to improve methods of chromosome doubling, both of which impact cotton genomics and breeding. This manuscript reports a procedure for cell cycle synchronization of root tips using hydroxyurea (HU), and the efficacy of several known chemicals for metaphase accumulation. Experimental treatments were administered hydroponically, followed by cytological determination of the metaphase index. An 18-hour treatment of HU at 3.5 mM gave a maximum synchronization of about 6%. Seedlings were treated with five antitubulin compounds of diverse chemistry; colchicine, amiprofos-methyl (APM), nitrous oxide gas, a benzamide designated RH-4032, and a novel phenylcyclohexene colchicine mimic RH-9472. Three of the antitubulin compounds, amiprofos-methyl, RH-9472, and RH-4032, provided average metaphase indices of 0.3 or higher on synchronized root tips and were preferable to colchicine, the standard agent for metaphase accumulation. The chromosome dispersing effects of nitrous oxide allowed the production of high quality chromosome preparations, although nitrous oxide showed little ability to increase metaphase indices or to act concertedly with the other mitotic blocking compounds, which tend to cause chromosome aggregation.

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