Enhancing Techniques for Studying Mitotic Peanut Chromosomes[1]

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ABSTRACT

Peanut chromosomes are small and preparations of well-spread, darkly stained mitotic cells can be difficult to obtain. Techniques developed for wheat were adapted to peanut. The results are highly reproducible and many dividing cells with darkly stained chromosomes can usually be observed when root tissues are used for preparation. Applications also can be made for determining ploidy levels and chromosome numbers of plants by using immature leaf tissues. The technique has application for both diploids and polyploids and is suitable for both chromosome counts and karyotyping studies.

Key Words: Cytology, cytogenetics, chromosomes, groundnut, *Arachis*.

Chromosome numbers in *Arachis* were first studied by Badami (1928) who reported a number of $2n = 20$ for *A. hypogaea* L. The true somatic chromosome number for the cultivated species was later observed to be $2n = 4x = 40$ by Kawakami (1930) and Husted (1931). Chromosome numbers for other *Arachis* species were first reported in the late 1940s for the tetraploid species *A. glabrata* Benth. (Gregory, 1946) and then for several diploids by Mendes (1947). Techniques used by the above-mentioned investigators were poorly adapted to peanut, and not until the report of Fernandez (1973) were many of the staining and tissue spreading difficulties overcome. He first pretreated tissues with paradichlorobenzene to collect metaphase cells, fixed tissues with a lactic acid-acetic acid solution, hydrolyzed roots in 1 N HCl and stained with aceto-orcein. However, cells in the proper stages of division for counting or karyotyping were relatively rare.

Several techniques have been used since the late 1970s to determine ploidy levels and to analyze peanut chromosomes. By correlating chloroplast number in guard cells, pollen grain size and ploidy level of peanut species, Singsit and Ozias-Akins (1992) reported a rapid method to determine the number of chromosome sets in *Arachis* genotypes. The most extensive chromosome studies, however, were by Stalker and Dalmacio (1981) and Singh and Moss (1982) who karyotyped diploid ($2n = 2x = 20$) species of section *Arachis*. Stalker and Dalmacio (1986) later karyotyped the two tetraploid species *A. hypogaea* and *A. monticola* Krapov. and Rigoni. Chromosome preparations by Stalker and Dalmacio were a modification of the technique used by Fernandez (1973), and adequate to perform their analyses; however, cells in the proper stage of division occurred at low frequencies. Similar problems with repeatability to obtain good preparations occurred in the laboratory of Singh and Moss (S. P. Tallury, pers. commun.).

In addition to repeatable results for chromosome preparations, nondestructive techniques are needed to identify the chromosome number in mature plants, especially after colchicine treatment of seeds or stems. The objectives of this paper are to report (a) a somatic chromosome preparation and staining technique which greatly improves the efficiency and quality of somatic chromosome preparations over previously reported techniques for peanut and (b) a nondestructive procedure to determine the chromosome number of peanut species and hybrids.

Materials and Methods

Seeds of five diploid ($2n = 2x = 20$) *Arachis* species (*A. rigonii* Krapov. and W.C. Gregory, GKP 10034, PI 262142; *A. appressipila* Krapov. and W.C. Gregory, GKP 9990, PI 261877; *A. glandulifera* Stalker, GKSSc 30091, PI 468336; *A. cardenasii* Krapov. and W.C. Gregory, GKP 10017, PI 262141; and *A. paraguariensis* ssp. *paraguariensis* Chodot and Hassl., KCF 11462) and *A. hypogaea* cvs. NC 4, NC 6, and NC 9 ($2n = 2x = 40$) were germinated in the laboratory by placing the seeds on the surface of moist vermiculite, with the radical end pushed into the medium. In approximately 1 wk the main radical and secondary roots were growing. The technique described below is essentially the same as the one described by Jauhar (1991) for wheat chromosomes, except that the pretreated concentrations are higher. One- to 2-cm lengths of the distal end of the roots were cut and placed in vials containing a solution of either 0.5 g/L colchicine plus 0.125 g/L hydroxyquinoline or 1 g/L colchicine plus 0.25 g/L hydroxyquinoline. The pretreatment was conducted at five 0.5-hr intervals ranging from 2 to 4 hr at room temperature. Roots were then washed in distilled water and placed into vials containing 2% aceto-orcein, again at room temperature. Roots were not fixed before staining. Vials were placed in a refrigerator (approximately 9 C) for at least 3 d, but storage for 1 wk or longer also gave good results. The stained roots were then placed in a vial containing 45% acetic acid, placed on a hot plate, and removed as soon as the solution started boiling. Roots became light-colored except for the root tip which remained dark; the darkly stained distal 1-2 mm of roots was removed and placed on a microscope slide, a drop of 45% acetic acid was added, and a cover slip placed over the root tip. If chromosomes were not well stained, a drop of aceto-orcein was added to darken them. Cells were then spread by tapping the cover slip over the root tip with a pencil and, with hand pressure, the chromosomes were squashed by pressing the slide which had been placed between two sheets of filter paper. Slides were made permanent by removing the cover slip using the dry ice method, dehydrating tissues in an alcohol series, and permanently mounting with Permount. The above technique also was used on roots of peanut stem cuttings for the seven genotypes. Immature leaves (1 cm or shorter) from *A. cardenasii* diploids and colchicine-treated plants also were collected. The basal 1 mm of leaf tissue at the point of petiole attachment was macerated, stained, and made permanent as described above. Chromosome counts were then made.

Results and Discussion

Mitotic chromosome preparations for both diploid *Arachis* species and *A. hypogaea* proved to be very good when using the described procedures for all genotypes studied. Best results for chromosome counting were obtained by using the pretreatment of 1 g/L colchicine plus 0.25 g/L hydroxyquinoline for 2 1/2 hr because chromosomes were both condensed sufficiently for accurate analyses and very darkly stained (Fig. 1a,b). This procedure is believed to have increased the meiotic index of dividing cells in late prophase to metaphase. If the acetic acid solution was not removed from the hot plate immediately after it started to boil, then chromosomes became lightly stained. Aceto-

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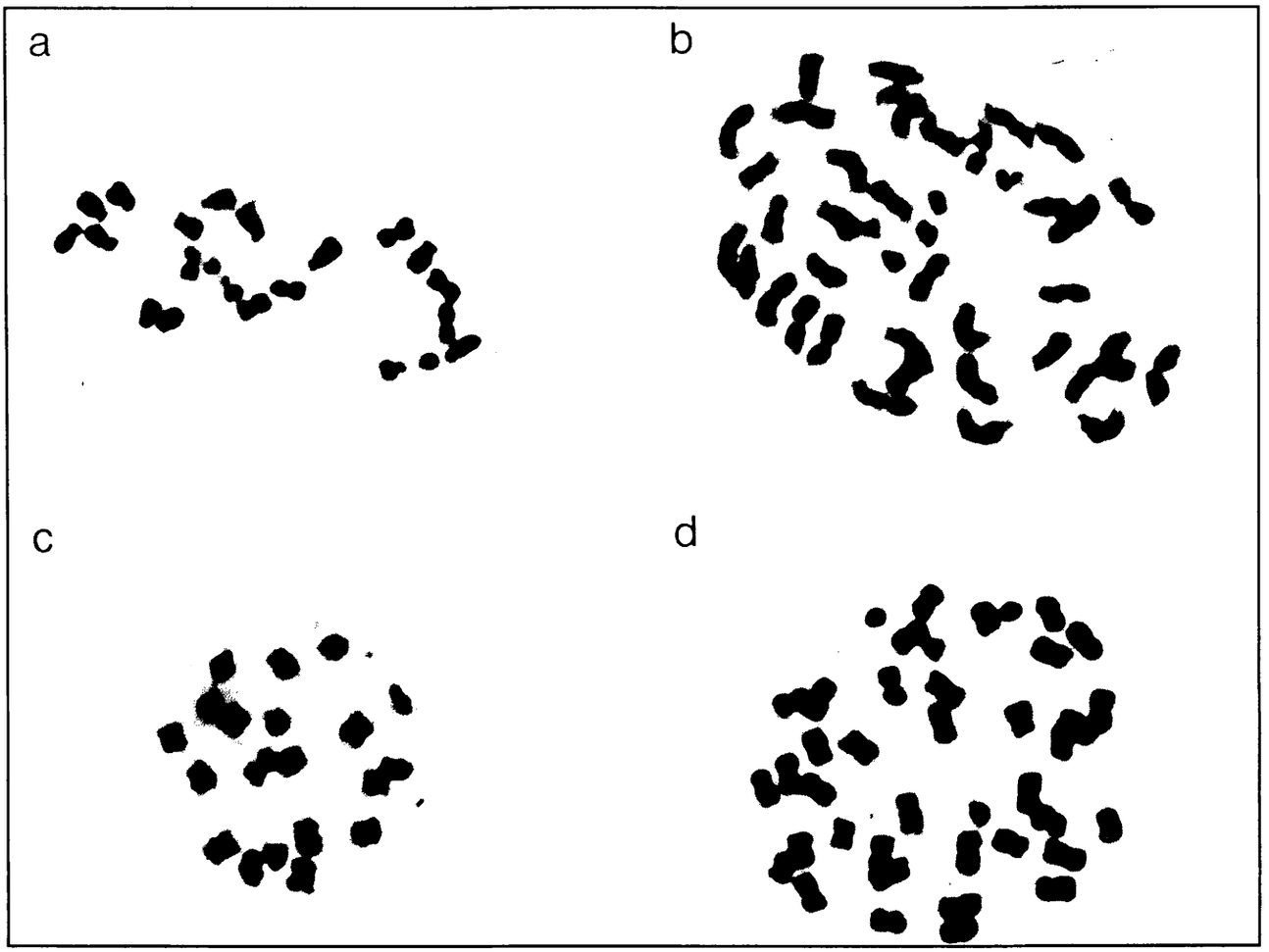

Fig. 1. Somatic chromosomes of *Arachis* where a = *A. glandulifera*, GKSSc 30091 ($2n = 2x = 20$) with moderately constricted chromosomes from root cells; b = *A. hypogaea* cv. NC 9 ($2n = 4x = 40$), with less constricted chromosomes from root cells; c = $2x$ *A. cardenasii* (GKP 10017) from leaf cells; and d = $4x$ *A. cardenasii* (GKP 10017) from leaf cells. All cells X5225.

orcein stain can be added beneath the cover slip to make chromosomes darker. The greatest improvement over previously reported techniques was the consistently high numbers of scorable cells (usually 20 or more per slide) which were spread sufficiently for relatively easy study. This is in contrast to fewer than one scorable cell per slide using the methods described by Stalker and Dalmacio (1981). Changing the pretreatment to 2 hr resulted in a higher frequency of less condensed chromosomes, which would be more useful for investigations involving karyotype studies of peanut.

Preparing root tips with a half-strength pretreatment (0.5 g/L colchicine and 0.125 g/L hydroxyquinoline) for 3 hr also gave a high frequency of good preparations of cells in early prophase. Preparations on some slides resulted in more darkly stained chromosomes, but the results were variable using this procedure. On the other hand, chromatids were more easily observed than when full strength pretreatments were used. Root tips collected from cuttings did not produce as many well spread and darkly stained preparations as did root tips from seedlings. Thus, a larger number of microscope slide preparations are needed for chromosome counts or karyotyping studies from cuttings.

Observations of macerated sections of tissues from the basal 1 mm of immature leaves, using the same procedures as for root cells, indicated that both the ploidy level and chromosome number can be determined from mature plants. The mitotic cells in division appeared smaller, however, and chromosomes were not spread as well as in root tips. Reliable chromosome counts of $2x$ (Fig. 1c) and $4x$ (Fig. 1d) plants could be made, although separation of aneuploid and euploid tissues would be more difficult than when root tips were used for analyses. The procedure is currently being used to identify autotetraploid and amphiploid plants because cuttings (to propagate root tissues) are often weak and die before adequate numbers of samples can be collected.

Several differences exist between the technique described here and ones previously described. First, Stalker and Dalmacio (1981) pretreated the roots in a saturated solution of paradichlorobenzene which does not constrict the chromosomes as greatly as hydroxyquinoline. They hydrolyzed the cells in 1N HCl rather than 45% acetic acid, which resulted in many cells being destroyed due to the harsh acid treatment. Singh and Moss (1982) used a modified carmine and feulgen technique which was also

rather unpredictable regarding numbers of scorable cells per root (S. P. Tallury, pers. comm.). The technique reported in this paper did not need a separate fixation step of root cells, which was achieved through placing the root tips in 2.0% aceto-orcein. The results obtained using the described technique for mitotic chromosome analyses are superior to other techniques used by the authors for obtaining large numbers of cells with well-spread and darkly stained chromosomes.

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