Cytogenetics in a *Kniphofia* hybrid

by Kaitlin Barrios

Introduction

The genus *Kniphofia* contains over 70 species of perennials with tall floral stalks bearing showy red, orange, yellow and or green tubular flowers. The common names of Red-hot poker and Torch lily stalks rise to tongues from spectaculling flame plants can be. In addition to the large number of species, there are at least 1,000 cultivars listed with more continuing to be released. Although mainly used in ornamental beds and borders, their spikes produce nectar which is a food source for birds. The flowers are pollinated by birds and insects and produce a large number of seeds. The seeds are black, flat and winged and second year and may green most of the year, producing the flower stalks in venture months. The vegetative portion of the plant ranges in height from 1.5' and the inflorescence can reach over twice that, depending of the species or cultivar (Whitehouse 2004). The genus has been known for quite a while, being first described in 1794 (Stephens, 1911). *Kniphofia* species are reported to hybridize fairly easily, making identification of these from the straight species very difficult (Moffett 1932).

The cytological research publications on this genus are not abundant and most are from at least 50 years ago. The most recent publication by Zonneveld et al. in 2005 compiled a list of nuclear DNA accounts for over 300 angiosperms using flow cytometry combined with many previous publications. Included in this study was one species of *Kniphofia* and one cultivar, *Kniphofia* 'Limelight' and *Kniphofia uvaria* (L.) Hook. f. (syn. *Tritoma uvaria* L.) with 2n=2x=14 and 10.60 pg/ but no ploidy level (Zonneveld et al. 2005). From my understanding, the researchers compared a sample of seed cultivar to one of *Agave americana* (internal standard) using flow cytometry to calculate the nuclear DNA content. At the individual chromosome level, Moffett characterized chromosomes observed in pollen grains and in root tips of several *Kniphofia* species and cultivars in 1932. Five of the six species or cultivar used for root cell chromosomal analysis were listed as 2n=12. The author also reported several plants had both diploid and haploid cells in the same root tip (Moffett 1932). Chromosome counts for *Kniphofia* species and cultivars have been reported as 2n=12, 2n=14, 2n=24, 2n=28 and 2n=32 (Moffett 1932). Chromosomes for *K. uvaria* (syn. *T. uvaria*) in 1980, another publication listed the ploidy and chromosome number for several *Kniphofia* species and varieties also as 2n=12, and at that time the family was listed as Liliaceae (De Wet 1980). Included in that report was the species *K. rooperi* (Moore) Lem which is thought to be in the hodgeneed, if not the parent, of our plant of interest.

As you stated earlier, many species of *Kniphofia* hybridize naturally, making identification difficult and even brings into question the existence of some species (Hongsuwan et al. 2015; Moffett 1932). Due to the nature of this common hybridization among species the ploidy and/or chromosome number is not commonly known. It is also not listed on the Royal Botanical Gardens Kew database of DNA C-values for *K. ulgbiella*. Therefore, we wanted to look into one of the parents of our plant, *Kniphofia rooperi* (syn. *T. rooperi*). Unfortunately, there is no information on the chromosome number or ploidy level for *K. rooperi* or a ploidy level, as well. We would also like to use flow cytometry to compare our plant with these plants if *K. rooperi* because it is thought to be in the genetic hodgeneed of our hybrid. Since the ploidy of *K. rooperi* is reported as diploid, flow cytometry would aid us to hone in on the ploidy of our plant.

Fig. 1

Photo of yellow form of *Kniphofia* sp. showing one giant multinuclear grain at 500x magnification (Moffett 1932).

Interpretive drawings (Moffett 1932)

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Materials and Methods

Plant Material and Collection

Plants of *Kniphofia* hybrid 'Harvest Moon' PPAF were utilized from Dr. John Stone's greenhouse at the USDA Dothan Horticultural Farm in Wetumpka, GA. Four healthy 5-gallon plants which had recently completed their reproductive stage were placed in 5-gallon pots bottom-filled with sand in mid-Feb. 2017 and acclimated under greenhouse conditions. Two healthy 5-gallon plants of *K. rooperi* which were utilized for flow cytometry were grown in the same greenhouse.

Root Tip Preparation

Root tips of *Kniphofia* hybrid 'Harvest Moon' PPAF plants were taken on several sunny mornings (~9am) following days of abundant sunlight during March and April 2017. The flowering process began with claiming root tips of sand and then immersing in *Acrylamycinpindica* for six hours at 4°C. Root tips were then transferred to Farmer's Karoice for approx. 18 hours at room temperature. After removing from fixative, root tips were placed in 70% ethanol at 4°C to store until staining and visualization of cells.

For visualizing chromosomes, root tips were first individually hydrolyzed in a 1:1 (v:v) solution of 95% ethanol and concentrated hydrochloric acid for approx. five seconds. The root was then placed on a glass microscope slide and the root tip (smaller than 1mm) was removed and dissected using a razor blade. A cap that slice of the stripped root and was then smeared, placed on the slide with interior section facing up, and the remaining root tip was discarded. Two drops of Feulgen stain were pipetted onto the root tip and allowed to stain for approximately 15 minutes. After staining, the root tip was squashed by manus of the sliding rotor blade technique and edge sealed with clear nail polish. The slides were then viewed using an Olympus BH2 fluorescent microscope under 40x magnification and images captured with an Olympus DP70 microscope camera and processed with Cell SensEntry software in Miller Plant Sciences on USDA Auburn campus. Serum cells were also photographed at 40 or 10x magnification using an Olympus BH2 microscope, DP25 digital color camera, and DP2-TWAIN program in the Parent lab at CAES on USDA Auburn campus. Some image files were resized using IrfanView software, Version 4.44.

Meiosis was counted using AceaSensitiplex 5.5 software.

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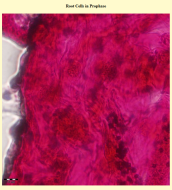
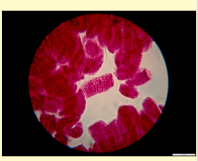
Results

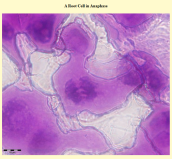
Slides Demonstrating Mitotic Phases

Root Cells in Prophase

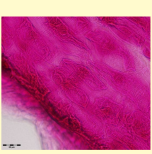


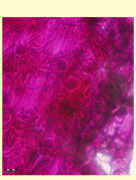


A Root Cell in Anaphase

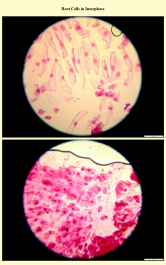


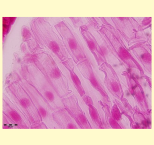
Root Cells in Putative Telophase



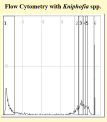


Root Cells in Interphase

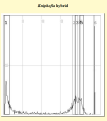




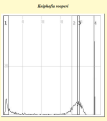
Flow Cytometry with *Kniphofia* spp.



***Kniphofia* hybrid**



Kniphofia rooperi



Combined *Kniphofia* hybrid and Presumed Hodgeneed Species

Conclusions

Results from flow cytometry strongly suggest that the ploidy level and nuclear DNA content of *Kniphofia* hybrid 'Harvest Moon' PPAF and *K. rooperi* are the same. And since *K. rooperi* is reported as 2n=12, we could infer that to be the ploidy and chromosome number of the cultivar 'Harvest Moon', as well.

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