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Polyploidy Induction by Colchicine Treatment in Kenaf (*Hibiscus cannabinus* L.)

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Summary

Polyploid breeding improves the ornamental value and efficiency of interspecific crosses. Kenaf (*Hibiscus cannabinus* L.) is expected to be a breeding resource for blue flowers among ornamental plants of the genus *Hibiscus*. We investigated the optimal conditions for polyploidy induction and the morphological changes induced by polyploidization in kenaf. The most efficient conditions for tetraploidy induction in blue-flower-type kenaf were soaking of the seedlings in 3.0×10^{-3} M colchicine solution for 24 h. Colchicine soaking at 1.0×10^{-3} M for 12 to 24 h was suitable for white-flower-type kenaf. In either type of kenaf, leaflet length to width ratio, guard cell length, petal length to width ratio, petal thickness, pollen diameter, and seed fresh weight were greater in tetraploids than in diploids.

INTRODUCTION

Some tropical and temperate species of the genus *Hibiscus* (Malvaceae family) are popular as ornamental plants because of their beautiful flower color and shape and their ease of cultivation. There is a wide range of flower colors, including white, yellow, peach, orange, and red, but few blue flower cultivars exist. Kenaf (*Hibiscus cannabinus* L.) is used mainly as a raw material for fiber, but it has not only white and yellow flowers but also blue flowers, which are rare among both wild species and horticultural cultivars of the genus *Hibiscus*. Therefore, kenaf with blue flowers is expected to be useful as a breeding resource to establish blue horticultural cultivars of the genus *Hibiscus*.

In interspecific crosses, sterility of the hybrid progeny is an obstacle to further breeding. Use of polyploids—mainly tetraploids—as hybrid parents can improve the success rate of interspecific hybridization and the fertility of the hybrid progeny. In this study, we tried to induce polyploids in kenaf as a preliminary step to interbreeding between kenaf and other *Hibiscus* species that are used as ornamental plants.

MATERIALS AND METHODS

Two strains of *H. cannabinus* L. were used (blue flower type and white flower type). Seeds were collected from plants grown in a greenhouse at the Gifu Field Science Center, Gifu University.

Seeds were dipped in sulfuric acid for 60 min for blue flower type and 30 min for white flower type to scarify the impermeable seed coat and then washed in running water for 1 h. They were then placed on moist filter paper in sealed plastic cases at 25 °C until germination. Germinated seeds with about 2 mm of root were subjected to polyploidy induction treatment with colchicine.

The seedlings were soaked for 12 to 48 h in 3.0×10^{-3} to 1.0×10^{-2} M colchicine solution containing 10% dimethyl sulfoxide. After the colchicine treatment, the seedlings were rinsed in running water for 1 h and then planted in plastic pots. They were grown under natural daylight in a glasshouse that was heated only in the winter.

Ploidy analysis was conducted by flow cytometry (Partec, Ploidy Analyser PAII, Germany). Fully expanded leaves were prepared for flow cytometry by using the chopping method (Galbraith et al., 1983).

Leaflet length to width ratio, guard cell length, petal length to width ratio, petal thickness, pollen diameter, and seed fresh weight were measured in diploids and tetraploids. Leaflet length to width ratio was measured in terminal leaflets. Petal thickness was evaluated from the ratio of petal fresh weight to petal area. Seeds were acquired through artificial pollination between plants of the same ploidy.

RESULTS AND DISCUSSION

Seedlings of blue-flower-type kenaf were treated for 24 or 48 h with 1.0×10^{-3}, 3.0×10^{-3}, or 1.0×10^{-2} M colchicine. Diploids, tetraploids, and octaploids, as well as chimeras in various combinations of 2x, 4x, and 8x, were observed after the colchicine treatment (**Fig. 1**).

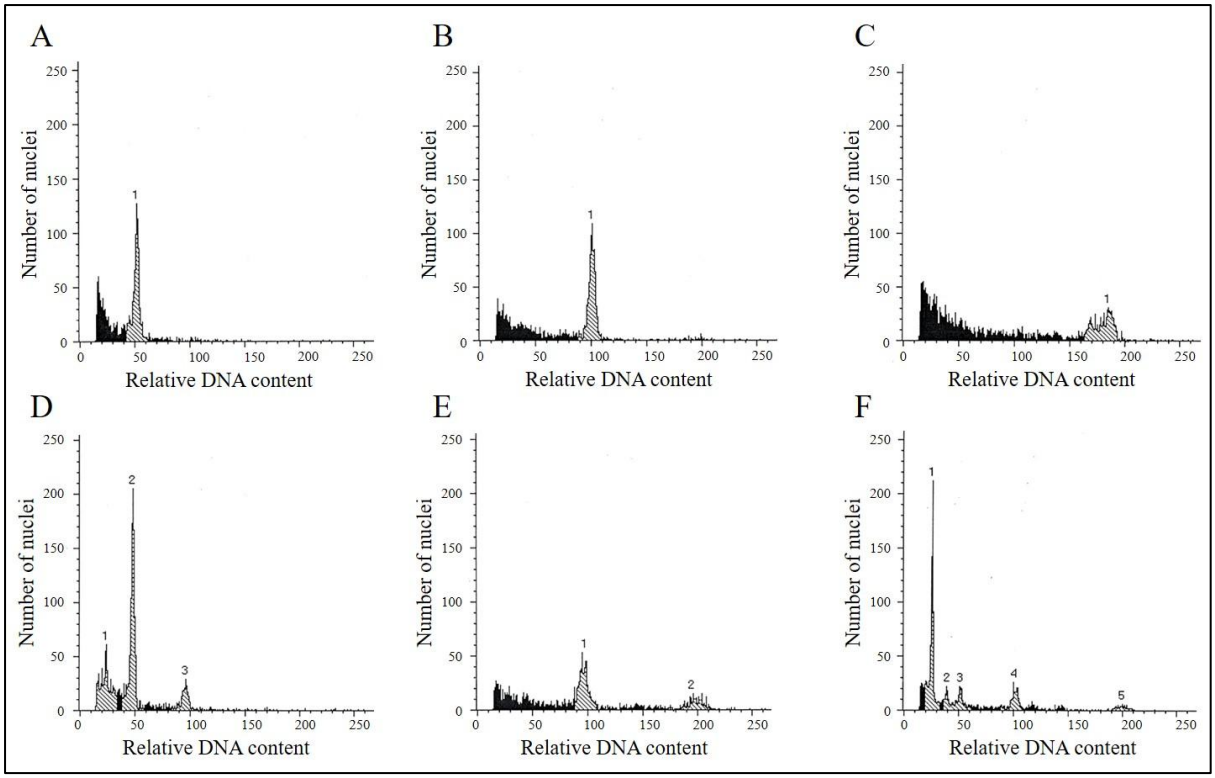

Figure 1. Histogram of flow cytometry after colchicine treatment in blue-flower-type kenaf. A) diploid; B) tetraploid; C) octaploid; D) chimera of 2x and 4x; E) chimera of 4x and 8x; F) chimera of 2x, 4x, and 8x. Numbered and shaded areas indicate estimated peaks.

RESULTS AND DISCUSSION

Tetraploids were obtained under all conditions (**Table 1**). About 300 seedlings were treated under each set of conditions. The greatest number of tetraploids was obtained with 1.0×10^{-3} M colchicine for 48 h, followed by 1.0×10^{-3} M colchicine for 24 h. The number of tetraploids decreased with increasing colchicine concentration. The numbers of other ploidy states were greater with 1.0×10^{-3} M colchicine than with 3.0×10^{-3} or 1.0×10^{-2} M.

The tetraploidy rate in surviving plants was highest with 3.0×10^{-3} M colchicine for 24 h, followed by 3.0×10^{-3} M colchicine for 48 h. The most efficient conditions for polyploidy induction in blue-flower-type kenaf were therefore 3.0×10^{-3} M colchicine for 24 h.

Based on the results in blue-flower-type kenaf, we tested a smaller range of optimal conditions for polyploidy induction in white-flower-type kenaf (1.0×10^{-3} or 3.0×10^{-3} M colchicine for 12 or 24 h). The survival rate was 48.4% or 34.4% with 1.0×10^{-3} M colchicine, and it was almost 0% with 3.0×10^{-3} M colchicine (**Table 2**). In the case of blue-flower-type kenaf, the survival rates were about 30% and 15%, respectively. High concentrations of colchicine inhibit root and shoot elongation and organ differentiation by inhibiting cell division. As we had observed no difference in germination rates between the two types of kenaf without colchicine in prior tests, we concluded that the white flower type was more susceptible than the blue flower type to the negative effects of colchicine.

The optimal conditions for polyploidy induction therefore differed within the same

species. Comparison with other *Hibiscus* species revealed that the optimal colchicine concentration for polyploidy induction in the two types of kenaf was higher than that for *H. mutabilis* L. (Ogasawara et al., 2010).

Table 1. Polyploid induction by colchicine in blue-flower-type kenaf.

Concentration of colchicine (M)	Duration of treatment (h)	Number of treatment	Number of survival	Number of 2x	Number of 4x	Number of other ploidy state	Survival rate (%)	Rate of 2x (%)[Z]	Rate of 4x (%)[Z]
1.0×10^{-3}	24	298	84	1	65	18	28.2	1.2	77.4
1.0×10^{-3}	48	300	95	9	80	6	31.7	9.5	84.2
3.0×10^{-3}	24	304	47	0	46	1	15.5	0	97.9
3.0×10^{-3}	48	283	43	0	41	2	15.2	0	95.3
1.0×10^{-2}	24	290	15	3	9	3	5.2	20.0	60.0
1.0×10^{-2}	48	294	44	2	34	8	15.0	4.5	77.3

[Z]Ratio of total number of plants to number surviving.

Table 2. Polyploid induction by colchicine in white-flower-type kenaf.

Concentration of colchicine (M)	Duration of treatment (h)	Number of treatment	Number of survival	Number of 2x	Number of 4x	Number of other ploidy state	Survival rate (%)	Rate of 2x (%)[Z]	Rate of 4x (%)[Z]
1.0×10^{-3}	12	64	31	0	27	4	48.4	0	87.1
1.0×10^{-3}	24	64	22	0	22	0	34.4	0	100.0
3.0×10^{-3}	12	64	1	0	0	1	1.6	0	0
3.0×10^{-3}	24	64	0	0	0	0	0.0	0	0

[Z]Ratio of total number of plants to number surviving.

We then compared the organ shape between diploids and tetraploids. Both the mature leaves and the petals of the tetraploids seemed wider than those of the diploids in the blue-flower-type kenaf (**Fig. 2**).

In fact, the leaflet width to length ratio and the petal width to length ratio were significantly greater in the tetraploids than in the diploids (**Fig. 3**). Petal thickness, pollen diameter, guard cell length, and seed weight were also significantly greater in tetraploids than in diploids.

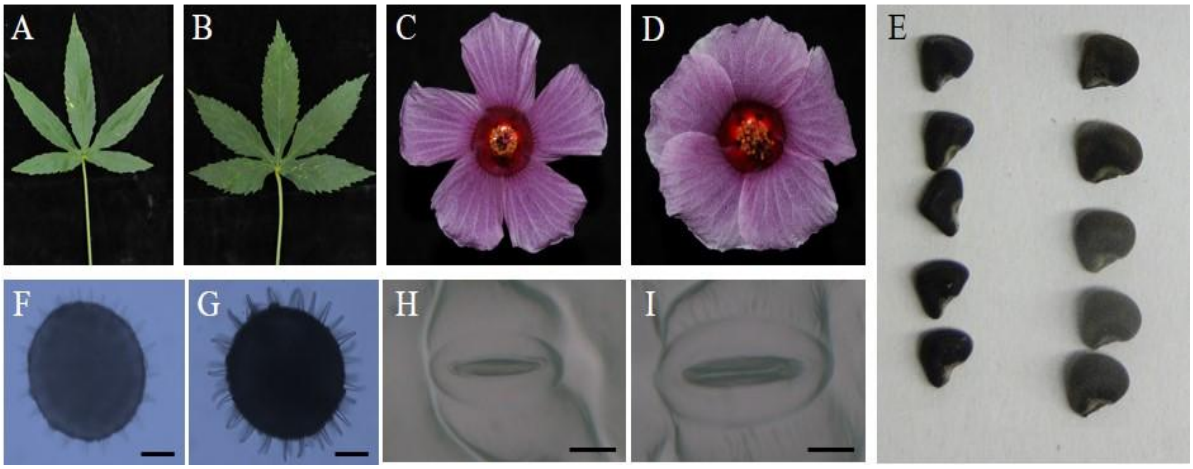

Figure 2. Characteristics of diploids and tetraploids of blue-flower-type kenaf. Mature leaves - A) diploid; B) tetraploid). Flowers - C) diploid; D) tetraploid. Seeds - E) left: diploid; right: tetraploid. Pollen - F) diploid; G): tetraploid, bars = 40 μm. Guard cells -H) diploid; I) tetraploid, bars = 10 μm.

Similar morphological changes were also observed in the white flower type (**Figs. 3** and **4**), and the trends and degrees of morphological change were roughly the same between the blue flower type and the white flower type. Increased organ width and larger size are common morphological changes caused by polyploidization in many plant species (Adachi et al., 2016; Niazian and Nalousi, 2020; Sugimoto et al., 2010), and these morphological changes were conserved in kenaf. In the genus *Hibiscus*, similar morphological changes with polyploidization have been observed in *H. moscheutos* L. and *H. mutabilis* L. (Li and Ruter, 2017; Ogasawara et al., 2010).

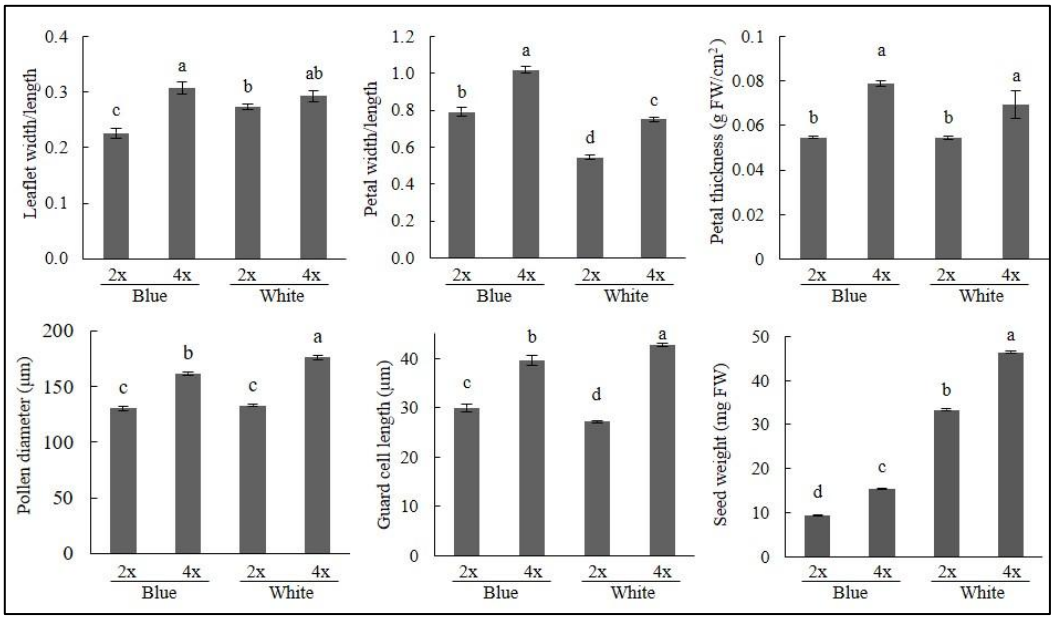

Figure 3. Characteristics of diploids and tetraploids. 2x: diploid; 4x: tetraploid; Blue: blue-flower-type kenaf; White: white-flower-type kenaf. Different lower-case letters in the same graph indicate a significant difference at $P < 0.05$ according to the Tukey–Kramer test.

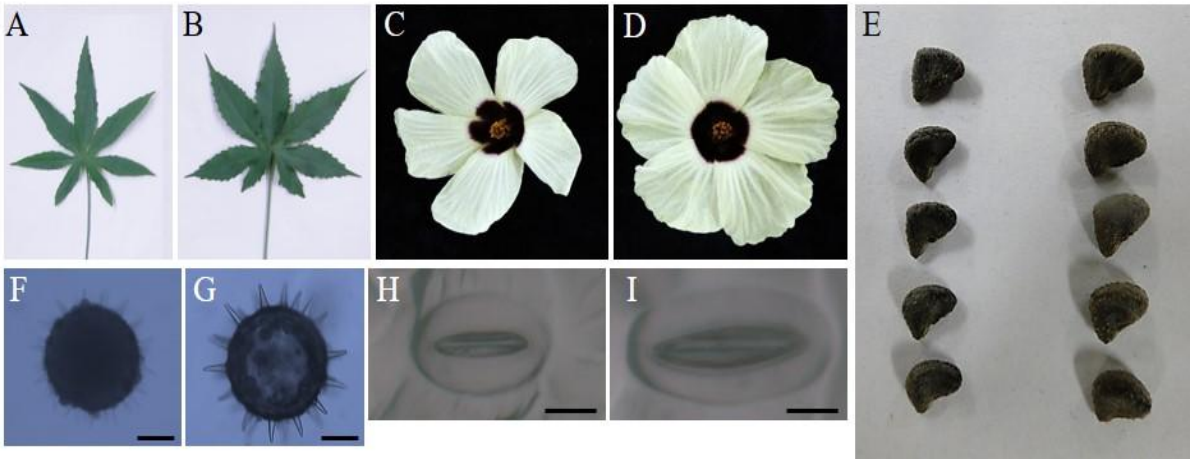

Figure 4. Characteristics of diploids and tetraploids of white-flower-type kenaf. Mature leaves - A) diploid; B) tetraploid. Flowers - C) diploid; D) tetraploid. Seeds - E) left: diploid; right: tetraploid. Pollen - F) diploid; G) tetraploid, bars = 40 μm). Guard cells - H) diploid; I) tetraploid, bars = 10 μm.

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