Induction and Identification of Tetraploids in *Taraxacum kok-saghyz*

Zinan Luo, Ron Fioritto, Catherine Freed, Nikita Amstutz, Sarah K. McNulty and Katrina Cornish

Department of Horticulture and Crop Science, The Ohio State University, Wooster, Ohio 44691

**ABSTRACT**

*Taraxacum kok-saghyz* (TK) domestication strategies include increasing the size and rubber content of this small dandelion species. Here, colchicine was used to induce tetraploids, of which eight successfully induced specimens were identified using flow cytometry. Initial evaluation indicates increased leaf width and stomatal size. Two 4-month old tetraploids had root rubber content twice the normal level of this age.

**INTRODUCTION**

*Taraxacum kok-saghyz* (TK) is an alternative rubber resource which has received considerable attention. One strategy to accelerate TK improvement is polyploid induction, which could increase plant size and the biosynthesis of chemical secondary products[1] such as rubber. Polyploidy is known to affect the accumulation of secondary products and increase the dimensions of plant structures (e.g. thicker stems, broader leaves, larger flowers, thicker roots, larger pollen grains and seeds) in many plants[2]. Therefore, chromosome doubling in TK may increase rubber yield. In the current study, the advanced testing technique of flow cytometry was used to ensure accurate ploidy determination following colchicine treatment.

**MATERIALS AND METHODS**

Colchicine Treatment

About 200 seeds were soaked in treatments with colchicine solutions of 0%, 0.05%, 0.1%, 0.2%, and 0.5% for 12h, 24h, 48h, 72h and 96h (Fig. 1). Seeds also were planted without colchicine treatment.

Visual Selection

The germination rate and survivorship were calculated at 1 and 4 weeks after treatment, respectively. Seedlings, including those with large, unusual leaf morphology (Fig. 2) were analyzed for ploidy using flow cytometry.

Flow Cytometry Analysis

Two months after treatment, the surviving seedlings and controls were analyzed using flow cytometer (Fig. 3). The induction rate was calculated and the analyzed seedlings were divided into three groups: tetraploids, treated (not successfully induced), and a control (untreated) group.

**RESULTS**

**Figure 1.** Germination, survival and induction rates were measured for each treatment. 0.2% at 24h was optimal.

**Figure 2.** Visual identification of seedlings. 80% of unusual leaves indicated tetraploids.

**Figure 3.** Flow Cytometric displays of (A) control (peak 1), 2n (peak 2); (B) 4n (peak 3); and (C) 2n + 4n chimeric plantlets. (B) illustrates desired tetraploids.

**Figure 4.** Different leaf morphology among the three groups.

**Figure 5.** Leaf morphological traits (length/width ratio, width and the number of leaves) among three groups. Tetraploids have wider, but shorter, leaves than diploids.

Sample roots excised from two 4-month old tetraploids were analyzed. Both contained 73 mg rubber/g dry root, double the diploid average.

**RESULTS (cont.)**

**Figure 6.** Comparison of stomata morphology under 40X microscopy. Tetraploid stomata (left panel) are clearly larger than diploid stomata (right panel).

**Figure 7.** Comparison of stomata morphological traits among three groups. Tetraploids have bigger but less stomata in the same microscopy area than controls.

**CONCLUSIONS**

- *T. kok-saghyz* seed were induced by colchicine and detected by flow cytometry. The optimal induction treatment was 0.2% colchicine for 24h.
- Visual selection guaranteed 80% of selected seedlings to be real tetraploids, making tetraploid induction and selection much more efficient.
- Obvious morphological traits can also be used to differentiate tetraploids from diploids in mature plants.
- 4-month old tetraploids had 2x rubber of diploids.
- A 4nx4n progeny has produced two viable seedlings.

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**REFERENCES**