Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
Instytut Nauk Ogrodniczych

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Wybrane aspekty określania stopnia mieszańcowości oraz mikrorozmnażania *Hippeastrum* sp.

The selected aspects of hybridity degree determination and micropropagation of *Hippeastrum* sp.

Rozprawa doktorska

Doctoral thesis

Rozprawa doktorska wykonana pod kierunkiem
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Warszawa, 2024

Abstract

Hipeastrum (*Hippeastrum* sp.) is one of the most widely cultivated geophytes, both for cut flowers and as a potted plant, and its popularity continues to grow. In this dissertation, research was undertaken to evaluate the morphological and genetic diversity of 15 clones and their parental forms from the SGGW breeding program launched in 2018, and to improve the method of micropropagation of *Hippeastrum*. The study included phenotypic evaluation, determination of chromosome number, ploidy level and genome size, determination of the number and length of stomata, examination of the degree of relatedness of clones and their parental forms (two clones of *H. ×chmielii* and three cultivars of *H. hybridum*) using RAPD and ISSR markers. In order to improve the micropropagation method, experiments were carried out on the effects of new growth regulators, including Karrikin 1 (KAR_1) and *Meta*-Topolin, as well as two selected biostimulants Goëmar Goteo and Agro-Sorb® Folium, on the performance of micropropagation itself as well as the acclimatization of plantlets and their subsequent cultivation. In addition, an analysis of the starch and fructan content in the bulbs of the parental forms and their progeny was carried out. Phenotypic analysis using the UPOV descriptor and the RHS color catalog made it possible to describe all 20 genotypes, as well as to select three promising clones: 0037-13, 0021-10 and 0023-11, which stand out relative to the maternal forms of *H. ×chmielii*. Studies using DAPI (4′,6-diamidino-2-phenylindole) and Feulgen's metaphase chromosome staining, and flow cytometry methods allowed us to determine the number of chromosomes and the ploidy level of the studied population. One diploid (2n = 2x =22), four triploids (2n = 3x = 33) and fifteen tetraploids (2n = 4x = 44) were selected. The genome sizes of the sample ranged from 32.63 - 62.23 pg. Analysis of the genome size of the 'Gervase' cultivar using a cytometer allowed for detection of a mixoploid form. Measurements of the length and number of stomata indicated that stomata could be a morphological marker of ploidy level. The use of RAPD and ISSR markers confirmed the high genetic similarity of the initial forms and progeny clones in the range of 74 - 85%. Biostimulants Goteo and Folium added to nutrient solutions in *Hippeastrum* micropropagation, affect plant weight growth, roots number, leaf and root length and can be an alternative to growth regulators. Goteo biostimulant, under plant acclimatization conditions, caused an increase in the quantity and length of roots, and after 7 weeks of cultivation, growth in plant weight and number of roots. Folium usage led to an increase in plant weight and quantity of leaves. KAR_1 significantly increased roots number in most *in vitro* and *ex vitro* genotypes. Application of KAR_1 during acclimation resulted in higher root number in nearly half of the cases compared to Goteo. *Meta*-Topolin added to the *in vitro* medium showed little effect on the bulb multiplication rate and markedly reduced root growth, while inhibiting the effect of KAR_1. Analysis of the starch content of the bulbs showed higher starch accumulation relative to both parental forms in 6 of the 15 progeny genotypes, and for fructans in 8 genotypes. The effect of KAR_1 on increasing sugar level in *Hippeastrum* bulbs was also demonstrated. The selected promising *Hippeastrum* clones could potentially become new Polish cultivars. The obtained results make it possible to increase the efficiency of the *Hippeastrum* propagation process by modern methods, to direct it to the use of biopreparations, and to improve the quality of the obtained planting material, including with increased content of reserve sugars.

Keywords: biostimulants, breeding clone, chromosomes, *ex vitro*, fructans, *in vitro*, karrikin, plant growth regulators, ploidy level, starch