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## ИНСТИТУТ ЭКОНОМИКИ И УПРАВЛЕНИЯ В АПК СЕКЦИЯ «ИНОСТРАННЫЙ ЯЗЫК В ПРОФЕССИОНАЛЬНОЙ СФЕРЕ И ЛИНГВОСТРАНОВЕДЕНИЕ

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# OPTIMIZATION OF PROTOPLAST ISOLATION TECHNOLOGY USING *DAUCUS CAROTA* (CARROT) LEAVES IN VITRO

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***Abstract:*** *In order to capitalize on the potential of using protoplast fusion technology to solve breeding difficulties within the framework of breeding programs for the creation of F1 carrot hybrids, factors impacting the qualitative and quantitative aspects of the protoplast isolation method were investigated. This work uses "Vilmarin" carrot leaves to isolate protoplasts through enzyme hydrolysis. Two main effectors—the concentration of sorbitol in preplasmolysis and enzymolysis time, affect the production and vitality of protoplast. The hemacytometer and FDA staining were used to determine carrot protoplast production and vitality, respectively.*

***Keywords:*** *Daucus Carota, Protoplasts, Enzymolysis Time, Preplasmolysis, Vitality, Viability*

## Introduction:

Carrot Daucus carota is an important root vegetable crop worldwide, with 42.83 million tons (carrot and turnips) production (FAOSTAT 2017). Carrots typically serve as a model plant in biotechnological techniques, in vitro culture has been well developed and employed into breeding programs to enhance productivity. Hence, new technologies must be developed through improving genotyping and

phenotyping methods and by increasing the available genetic diversity in breeding germplasm [1,2,3].

The advancement of protoplast technology has received much attention and has made tremendous progress. If a reliable and efficient plant regeneration system could be built from isolated protoplasts then genetic manipulation by protoplast technologies such as somatic hybridization, Cybridization, or direct gene transfer may be employed for plant improvement [4].

In this paper, the parameters of protoplast isolation were assessed, including sorbitol concentration for preplasmolysis and enzymolysis time using carrot leaves. At long last, we examined how these variables affected the viability and yield of carrot protoplasts. This study made a fundamental report on the getting ready states of protoplasts, which can establish the groundwork for genetic programs and breeding of new varieties.

**Materials and methods**

"Vilmarin" carrot line from the collection of N.N. Timofeev Breeding station were used as protoplasts donor. Aseptic material was derived from seeds sterilized using a three-step procedure. First, incubation seeds in a water bath at 50˚C, then surface sterilized. Finally, rinsing 3 times with sterile distilled water. The seeds were sown on 9 cm round Petri dishes containing solid Murashige and Skoog 1962 (MS) medium [5] with vitamins supplemented with 30 g/l sucrose and 6.5 g/l plant agar and maintained at 24 ± 1°C in the dark for 7 days. culture seedlings were transferred to Petri dishes containing regeneration medium composed of MS macro- and micro-elements, 0.1 mg/l thiamine HCl, 0.1 mg/l pyridoxine HCl, 0.5 mg/l nicotinic acid, 3.0 mg/l glycine, 100 mg/l myo-inositol, 20 g/l sucrose, and 2.5 g/l phytagel. Cultures were kept in the climate room at 24±1˚ C under 16 h photoperiod.

***Protoplast isolation:***

Protoplasts were isolated from leaves with petioles from 5-week-old carrot plantlets using the protocol of Baranski et al. (2007) [6], with special modifications. In details, about 1 g of tissue was placed in a glass Petri dish and cut into fine pieces with 8 ml of preplasmolysis solution (pre-treatment phase) with different sorbitol concentrations (0.3, 0.5 and 1 M sorbitol with 0 M as control + 0.05 M CaCl2.2H2O), replicated three times and incubated for 1 h in the dark at 24 ± 1C (figure 1).

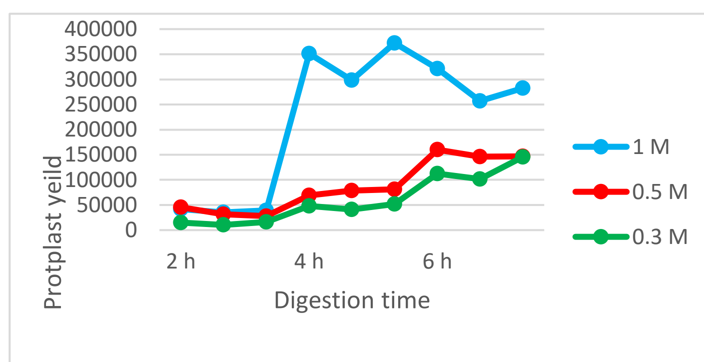


**Figure 1 The Effect of sorbitol concentration on the yield of protoplasts.**

To optimize the enzymolysis time, the tissues were digested for 2, 4 and 6 h at 24 ± 1C in the dark with gently shaking (30 rpm) in enzyme mixture consisting of 1% (w/v) cellulase Serva, 0.1% (w/v) pectinase Rohament p5, 20 mM 2-(N Morpholino) ethanesulfonic acid (MES, Panreac), 5 mM CaCl2.2H2O, and 0.6 M mannitol, pH 5.6, filter-sterilized (0.45 µm, Millipore). Then, protoplasts were filtered through nylon membrane (100 µm and then 40 µm respectively) the remaining pieces of leaves in the dish were mixed and squeezed to petri dish walls with 0.5 M mannitol-D (Dia-M) solution and filtered to obtain more protoplasts, which then centrifugated at 150 rcf for 10 min at room temperature in a swinging bucket rotor, the supernatant was discarded. The precipitation at the bottom of the centrifuge tube were the protoplasts, which were washed two times in MMG containing 4 mM MES buffer (pH 5.7), 0.6M mannitol and 15mM MgCl2.

**Results and Discussion:**

Comprehensive protoplast yields viability and other considerations, to determine the optimum conditions for 5-week-old carrot mesophyll protoplast separation. Based on the results, 0.5 M sorbitol pre-treatment for one hour, the combination of 1% (w/v) cellulase, 0.1% (w/v) pectinase and 6 h incubation time were the most suitable conditions for protoplast isolation of D. carota using in vitro leaves in the study. The established protocol could be applied in future studies on somatic hybridization and protoplast fusion.

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# EVALUATION DE LA MINERALISATION DE LA MATIERE ORGANIQUE DANS LES AGREGATS DES SOLS SOUS LES DIFFERENTS TYPES D'ITULISATION DES TERRES

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