the information given and the statements subsequently start to be receiving more transparently and confidentially. Apparently, the speaker is using the strategy of *resonation with the target audience,* which can be explained basing on the fact that the main goal of the interview was to provide the audience with all the information about the qualities and new up-to-date functions of the phone.

Overall, as is seen from the analysis done, combination of language means of different order, level and style, that are discovered in PR discourse material, are undoubtedly involved in creating effective professional communication.

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УДК 631.52

CARROT PROTOPLAST ISOLATION AND FUSION WITH RELATIVES: A KEY TOOL FOR BIOTECHNOLOGICAL AND PLANT BREEDING RESEARCH

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***Abstract.** Protoplast fusion is a novel tool for transferring genes for a desired quality and quantity of production. In this technology, two different genetically originated protoplasts from different somatic cells are fused in order to obtain hybrid protoplasts [4]. In our case obtaining male sterile CMS carrot lines by protoplast fusion, which considered the main method used in carrot breeding. Cytoplasmic male sterility (CMS) is commercially utilized for hybrid seed production.*

***Keywords:** Daucus carota, protoplast fusion, hybrid, cytoplasmic male sterility CMS, Aloplasmics, self-pollination, self-incompatibility.*

Introduction: In terms of production areas and market value, carrot ranks among the top ten most economically significant vegetable crops worldwide.

There are two primary types of cytoplasmic male sterility (CMS) in carrot: earthy colored anther" and "petaloid". The plant trait cytoplasmic male sterility (CMS) is determined by the mitochondrial genome and is associated with a sterility phenotype in the pollen. Nuclear genes known as restorer-of-fertility genes can either suppress or counteract this phenotype [1].

F1 hybrids in carrot are valuable for their uniform maturity, high early and total yield, better curd quality in terms of compression and color, and resistance to insect pests, diseases, and adverse weather conditions. However, an effective, dependable, and established method of producing F1 seeds without infectivity using self-fertilized seeds from each parent is essential. Due to the incompatibility of the flower's size and structure, the manual emasculation and pollination method for carrot is unsuitable for commercial purposes. The self-incompatibility (SI) system has been utilized in the creation of hybrid carrots thus far. Hybrids created with the CMS system for yield, yield-linked, and quality traits have also shown significant heterosis in recent years [3].

CMS, caused by mutations in the mitochondrial genome, is found in higher plants and increases heterosis and improve genetic resources. Mitochondrial markers can be used to differentiate between the various types of CMS sources. Molecular markers that can predict the CMS status at an early developmental stage will be valuable tools in carrot breeding and seed production programs, as well as for basic studies of male sterility [2].

Materials and methods

Last year, 1 fennel hybrid and 4 hybrids of celery, were ordered and cultivated in order to select MS relatives of carrot. Since celery is biannual crop, only this spring has bloomed in the greenhouse, the flowers of these hybrids have been examined visually, microscopically and molecularly to check the MS trait within them.

Molecularly: DNA of celery and fennel have been isolated to carry out the PCR which is followed by gel-electrophoresis to check the existing of carrot CMS within their genomes using molecular markers.

Visually: every plant was estimated in a visual manner for pollen grains and anthers of their flowers.

Microscopically: seeing via microscope the existing of pollen grains.

Plant material:

Carrot and fennel seeds were surface sterilized in 70% ethanol for 5 min, followed by 15 min in 0.75% NaOCl with 2 drops tween 20, they were then rinsed 3 times in sterile distillate water and sown in Petri dishes containing MS medium with vitamins supplemented with 20 g/l sucrose, 25 mg/l NaFeEDTA, 2 mg/l glycine and 6 g/l plant tissue culture agar, and incubated at 22˚ C in the dark for germination and plantlet growth. 7 days old seedlings were transferred to the same solid medium [5].

Callus induction and cell suspension initiation:

Leaf and petiole explants were cultured on MS medium supplemented with 30 g/l sucrose, 0.5 g/l enzymatic casein hydrolysate, 0.5 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), 0.5 mg/l kinetin and 7 g/l agar. The cultures were maintained at 22±2 °C in the dark and friable callus was selected and refreshed monthly.

The cell suspension cultures were initiated by culturing 250 mg of friable callus in Petri dishes containing liquid suspension medium based on MS medium supplemented with 30 g/l sucrose, 0.5 g/l enzymatic casein hydrolysate, 0.6 mg/l 2,4-D and 0.55 mg/l kinetin. The cultures were incubated at 22±2°C in the dark with continuous agitation (100 rpm) [5].

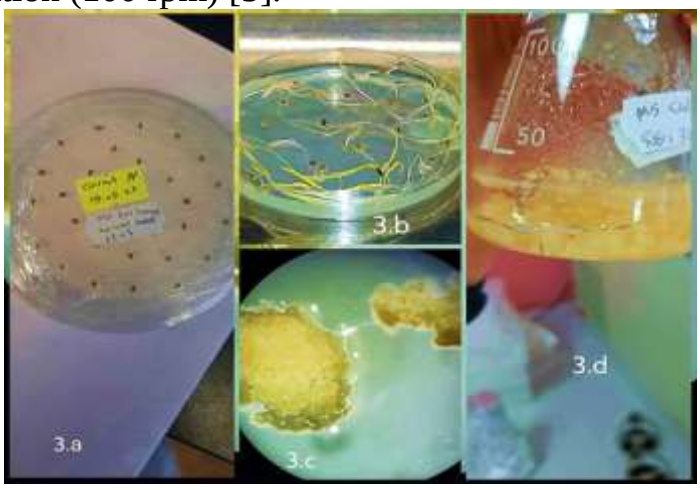

Fig1 a. carrot seeds after sterilization and cultivation in Petri dishes, b. carrot seedlings, c. callus formation, d. stabilized callus suspension

Protoplast isolation and purification:

Protoplasts were isolated from 5- to 9-week-old suspension cultures at the 4th day after subculturing using the protocol of Grzebelus et al. (2012), with some modifications. About 1 g fresh weight of suspension cells was incubated in 10 ml enzyme solution that contained 0.5% (w/v) cellulase, 0.05% (w/v) pectolyase, 20

mM MES, 5 mM CaCl2, and 0.6 M mannitol. The digestion was performed overnight at 22 °C by gently shaking (30 rpm) in the dark. After digestion, the protoplasts were subsequently sieved through 100 µm (Falcon) and 40 µm (SPL Life Sciences) nylon sieves, washed with 15 ml of W5 medium and centrifuged at 100 g for 5 min. The protoplasts in the pellet were resuspended in 10 ml of 0.6 M sucrose on top of which 1 ml W5 medium was overlaid. The samples were centrifuged at 80 g for 10 min, and the viable protoplasts localized in the interphase between the two solutions were collected and subsequently washed in 10 ml W5 medium and 10 ml culture medium and centrifuged at 100 g for 5 and 10 min [5].

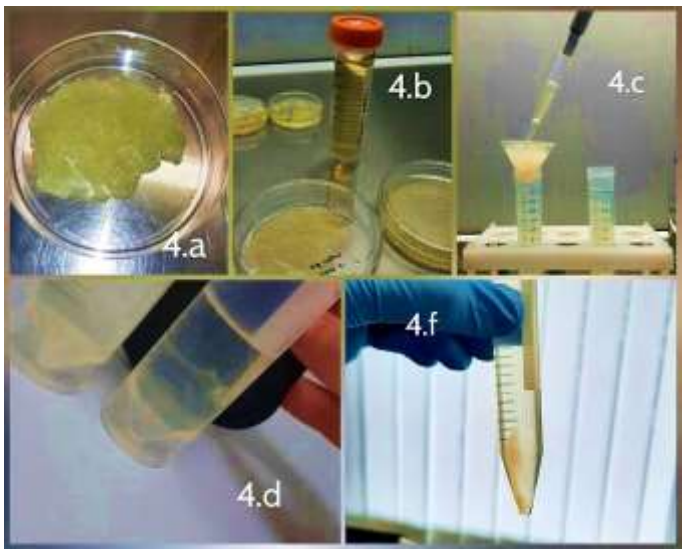

Fig 3. a. fresh cell suspensions, b. adding enzyme solution, c. filtration, d. interphase between two solutions, f. pellet formation after centrifugation

Results and Discussion:

Molecularly: the electrophoresis results showed that celery, neither fennel hybrids have the same kind of male sterility which carrot has, this is a good induction principally.

Visually: 3 hybrids of celery are sterile (Mambo, Seinnia and Balina) as well as fennel hybrid (Dragon)

Microscopically: it proved the same points of visual results; thus, we can use those sterile hybrids as protoplasts donners.

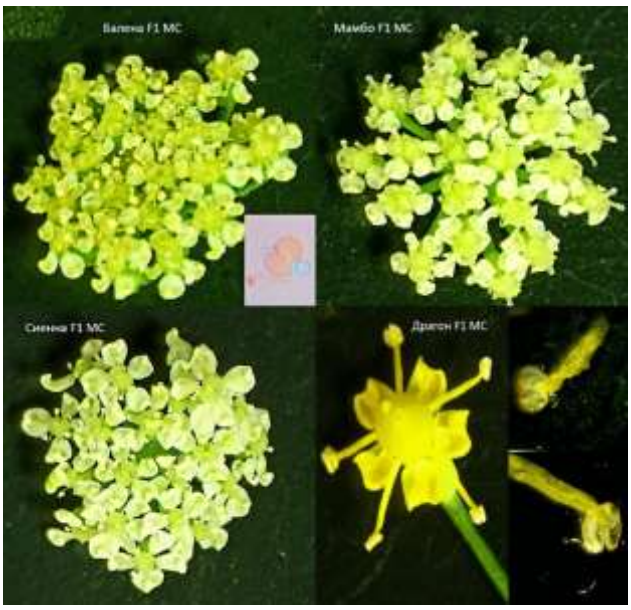

Fig 2 Sterile flowers

Protoplasts were acquired, but at a low concentration, insufficient for fusion.

Notably, healthy protoplasts were obtained from a 40 μm filtered callus suspension, as this allows the passage of protoplasts without other cells or impurities.

The same process will be repeated for celery and fennel seeds; Protoplast fusion will be carried out using the Gene Pulser Xcell electroporation system to obtain alloplasmic protoplasts.

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