EXPERIMENTS AIMED AT THE INDUCTION OF SOMATIC EMBRYOGNESIS WITH TOMATO (SOLANUM LYCOPERSICUM L.) AND CAULIFLOWER (BRASSICA OLERACEA CONVAR. BOTRYTIS VAR. BOTRYTIS L.) GENOTYPES FOR THE PURPOSE OF ARTIFICIAL SEED PRODUCTION

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The production of hybrid seed used in modern crop cultivation has become increasingly challenging due to difficulties in controlled pollination, extreme fluctuations in climatic conditions, and various technical, economic, and regulatory hardships. With advancements in biotechnology, opportunities are arising to improve and alleviate or overcome these limitations. Tomato (*Solanum lycopersicum* L.) and cauliflower (*Brassica oleracea* convar. *botrytis* var. *botrytis* L.) are both economically important vegetable crops that have been widely studied in the field of plant tissue culture.

These techniques allow the in vitro maintenance, regeneration, and propagation of plant cells and tissues. The aim of our research was to develop an efficient methodology using these tissue culture techniques to induce callus and produce propagation material suitable for potential artificial seed production. During our investigation, we focused on finding answers to the following questions:

- which plant-derived explant (hypocotyl, cotyledon, or first leaf) performs the best within a given genotype, in terms of callus induction, organogenesis, or somatic embryogenesis,
- to identify the most suitable combination of callus induction and somatic embryogenesis-inducing media,
- which media combination results in successful somatic embryogenesis,
- which medium combination induces organogenesis,
- what regenerative capacity is showed by calli maintained in liquid culture?

The plant material used in this study was, in the case of tomato: ‘Bobcat F1’ (P1), ‘Dominet’ (P2), ‘Hapynet’ (P3), and ‘Sagatan’ (P4). And in the case of cauliflower: ‘Amidala’ (K1),

‘Andromeda’ (K2), ‘Clarina’ (K3), and ‘Clementine’ (K4). To achieve our objectives, two plant growth regulators were tested in callus-induction media.

All three explant types for each genotype were cultured on three media variants: the “2” medium containing 2 mg/l 2,4-D and 0,5 mg/l BAP; the “4” medium containing 4 mg/l 2,4-D and 0,5 mg/l BAP; and an MS control medium without growth regulators. The results clearly demonstrated genotype- and explant-dependent differences in callus growth rate, with cotyledon explants generally showing a higher proliferation rate than other tissues. Tomato genotypes typically showed greater callus growth rate on the medium “2” than on the medium 30 with the higher auxin concentration (4 mg/l), while a similar trend could not be observed for the cauliflower genotypes, which could only be assessed genotype by genotype.

In the next step, calli were transferred to somatic embryogenesis-inducing media. The composition of these were the following: MS + 0,05 mg/l IAA + 0,5 mg/l kinetin + 2% sucrose and MS + 3,5 mg/l BAP + 0.5 mg/l IAA + 3% sucrose. Although the formation of somatic embryos was not observed on any of the media combinations, organogenic tissue induction occurred in all cauliflower genotypes on the latter embryogenesis-inducing media. The highest number of shoots was recorded in the ‘Clarina’ (K3) genotype, which was then selected for our artificial seed encapsulation experiments.

The grown shoots of the ‘Clarina’ (K3) genotype were encapsulated in both a growth regulator-free MS and a root-inducing medium based on MS salts containing half-strength macronutrients, supplemented with 2 mg/l BAP, 1 mg/l TDZ, 1 mg/l TOP, 0,1 mg/l IBA, 2 ml/l WUXAL® Super, and 500 mg/l MES. For gel formation, 15 g/l sodium alginate and 50 mM $CaCl_2$ were used. Then the MS-based beads were put on solidified MS media, and three of the root-inducing ones were immediately planted into sterile peat pellets, while the remaining capsules were stored at 4 °C for 5, 10, and 20 days before planting. The encapsulated shoot apices were maintained under sterile conditions, and although shoot growth began in both types of capsules, root growth was not observed in either. We could not observe differences between the groups of the stored capsules.

The tomato and cauliflower genotypes were also cultured on media containing 4 mg/l NAA, which induced callus formation, only in the cauliflower genotypes and organogenesis in both. Root development was observed on cotyledon and hypocotyl explants across all genotypes, while shoot formation occurred only in cauliflower. In tomato genotypes, “Rhizoid Tuber” like structures could be observed, previously described as embryogenic formations by Saeed et al. (2019). These structures were transferred to an embryo maturation medium (MS + 5 mg/L BAP

+ 2% sucrose), where greenish tissue growth, characteristic of early embryogenic development, appeared by the third week, but maturation was not reached.

The initial callus suspension cultures, established in liquid versions of the callus induction media, showed slow proliferation. Even after periodic subculturing, no significant callus growth or embryogenic induction was observed when the cells were reintroduced onto solid media. One exception was noted in the cauliflower genotype ‘Clarina’ (K3), where organogenic callus 31 developed, forming both roots and shoots. Calli derived from the 4 mg/l NAA-containing solid MS B5 media of the ‘Dominet’ (P2), ‘Sagatan’ (P4), ‘Clarina’ (K3), and ‘Clementine’ (K4) genotypes were transferred to liquid culture containing 10 mg/L NAA. The ‘Clarina’ (K3) calli originating from cotyledon and hypocotyl tissues showed a visibly high proliferating response and root formation, with the shoot apex also emerging. A similar but less pronounced response was observed for the ‘Clementine’ (K4) genotype. In contrast, tomato genotypes did not produce viable callus tissues in liquid culture.