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Isolated microspore culture in eggplant and inducing heart-shaped embryos

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ABSTRACT

In many crops, androgenesis is one of the most common methods for *in vitro* haploid induction. The most effective technique in this system is the isolated-microspore culture, in which haploid cells are reprogrammed to follow the sporophytic pathway. But this pathway has been stopped at the globular shape stage of embryogenesis in eggplant, and efforts are still being made to overcome this problem. In this study, the effects of different concentrations of gum arabic, sucrose, and plant growth regulators (6-Benzylaminopurine, BAP, and 1-Naphthaleneacetic acid, NAA) were evaluated on isolated microspore cultures from two cultivars of eggplant. In cultivar Ricarda, the highest number of microspore-derived calli (711.4 per Petri dish) was produced when 2000 mg/l gum arabic, 2% sucrose, 0.5 mg/l BAP, and 0.5 mg/l NAA were used together. By combining 2600 mg/l gum arabic, 2% sucrose, and 0.5 mg/l BAP and NAA, the cultivar Chantale produced the most calli (230.33 per Petri dish; 5.27-fold higher than the control (43.73 per Petri dish). In addition, the results showed that heart-shaped embryos could be produced in eggplant. The culture of microspores of cultivar Ricarda in NLN medium supplemented with 2600 mg/l of gum arabic, 2% sucrose, 0.5 mg/l BAP, and 0.5 mg/l NAA led to the developmental progression of some of the globular structures. In fact, the globular embryos were induced to develop into heart-shaped embryos, which is a promising step forward in the process of eggplant microspore embryogenesis.

Key words: BAP, Gum arabic, Microspore callogenesis, Microspore embryogenesis, NAA, Sucrose.

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INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the 1300 species present in the Solanaceae family (Knapp *et al*., 2019). Eggplant was the third largest vegetable produced in the world in 2018, after potatoes and tomatoes, with a production of 55.2 million tons in 1.85 million hectares. China, India, Egypt, Turkey, and Indonesia are some of the most important eggplant-producing countries (37.4, 12.9, 1.3, 0.8, and 0.7 million tons, respectively) (FAOSTAT, 2021). The breeding efforts have been focused on developing high-yielding eggplant varieties, often F1 hybrids. Heterosis has been used to improve different traits in eggplant, such as long-term viability, uniformity, pest and insect resistance, and better growth (Kumar *et al*., 2020). Doubled haploid technique is the fastest way to generate new inbred lines, and this technique plays a significant role in breeding programs. Androgenesis and gynogenesis are two terms for the *in vitro* culture of male and female gametes, respectively. These techniques allow the main gametophytic pathway to be changed to a sporophytic pathway, within which haploid plantlets can be produced. Androgenesis (anther culture and isolated microspore culture) is the most common method for many crops to produce doubled haploids (Vural and Ari, 2020). Isolated microspore culture has attracted a lot of attention because of the problems caused by the anthers' walls, which induce diploid somatic callus and block nutrients that microspores need to develop. This is also because of the limitations for developmental and tracking studies about how microspore embryos grow and mature (Ferrie and Caswell, 2011). The eggplant microspore culture was reported for the first time by Gu (1979). He showed that four days of anther pretreatment on solid MS medium with different treatments, including a medium supplemented with 2 mg/l dichlorophenoxyacetic acid (2,4-D), 1 mg/l kinetin, 3% sucrose and 0.8% agar, before pollen isolation was useful for dividing pollen grains and forming the callus. This report showed that the best medium for pollen culture included the macroelements of MS medium, 2 mg/l 2,4-D, 1 mg/l kinetin, 800 mg/l glutamine, 5 g/l myo-inositol, and 100 mg/l serine. Then, Miyoshi (1996) could develop a documented method for the microspore culture of eggplant. This method was based on suspending microspores in their late uninucleate and early binucleate stages in a starvation medium (distilled water) and incubating them at 35 °C for three days under dark conditions. The microspores were then transferred to liquid NLN medium (Lichter, 1981) without potato extract with 2% sucrose, 0.5 mg/l of benzyl adenine (BA) and 0.5 mg/l of NAA. This procedure led to the formation of callus-like structures with different ploidy levels and low regenerative ability, but no embryos were produced. However, eggplant microspore culture was resumed via modifying tobacco microspore culture protocol by Bal *et al*. (2009), but the results only showed symmetrical divisions and multicellular structures. Corral Martinez and Seguí-Simarro (2012, 2014) then modified Miyoshi's method (Miyoshi, 1996). They observed that the obtained embryos from the eggplant microspore culture stopped in the globular stage and deviated toward the callus formation pathway. Their results also showed that adding polyethylene glycol, epi-brassinolide, and gum arabic can help improve the efficiency of embryogenesis and callus induction. In another study, the effects of some treatments on eggplant microspore embryogenesis were investigated both *in vitro* (using different concentrations of NLN components, plant growth regulators, sucrose, and different microspore culture densities) and *in vivo* (using higher levels of boron and exposing donor plants to different temperatures and light levels). The intensity of light hitting the donor plants increased the viability of the eggplant microspores (Rivas-Sendra *et al*., 2020). In another study, the levels of endogenous plant growth regulators (cytokinins, auxins, gibberellins, jasmonic acid, abscisic acid, and salicylic acid) were investigated in eggplant microspore embryogenesis. During microspore culture, different patterns by which plant growth regulators accumulate are linked to different embryogenic responses (Calabuig-Serna *et al*., 2021). Even with all these efforts, developing eggplant globular embryos into later embryonic stages is still a great challenge.

Gum arabic is composed of three fractions with similar proportions of various sugars which differ principally in their molecular masses and nitrogen contents: arabinogalactan (AG) fraction (88.4% of the total weight, and low in protein (0.44% w/w)), arabinogalactanprotein (AGP) fraction (10.4% of the total weight and with a greater proportion of protein (9.18% w/w), and glycoprotein (GP) fraction (ca. 1% of the total weight, and with high protein content (ca. 50% w/w) (Vandevelde and Fenyo, 1985; Randall *et al*., 1989). Gum arabic contains approximately 37% galactose, 14% rhamnose, 26% arabinose and 16% glucuronic acid. Proteins obtained from gum arabic (*Acacia senegal*) contain 17 amino acids, among which hydroxyproline, serine, proline, aspartame, thyronine and leucine are the most abundant (Osman *et al*., 1993). Gum Arabic contains also some mineral

elements including K, Na, Ca, and Mg (ElAmin *et al*., 2015). Arabinogalactan proteins (AGPs) are rich in carbohydrates and present in the cell wall (Ma and Johnson, 2021). Morphogenic processes such as somatic and microspore embryogenesis can be affected by AGPs. The positive effect of AGPs has also been demonstrated exogenously on increasing microspore embryogenesis. AGPs may be signaling or potential regulatory molecules in microspore reprogramming, but the information is still very limited on the synthesis and location of AGPs and their role in microspore embryogenesis (Zielińskia *et al*., 2021). AGPs are effective in forming and promoting the initial pattern of microspore embryogenesis in wheat (Letarte *et al*., 2006), maize (Borderies *et al*., 2004), rapeseed (El-Tantawy *et al*., 2013), barley (Makowska *et al*., 2017), and bell pepper (Pourmohammad *et al*., 2021).

Sucrose is an important source of carbon and energy in *in vitro* culture, and can also act as an osmotic agent by affecting somatic embryogenesis in different species (Shi *et al*., 2009; Lema-Ruminska *et al*., 2014; Mishra and Singh, 2016). Microspore survival may be affected by sucrose concentration. A study of microspore survival and embryo induction in rapeseed revealed the highest survival (after 16 d) and the greatest number of anthers with induced embryos (after 42 d) occurred on the highest sucrose concentration (20%). However, macroscopic embryos emerged only from anthers on 8% sucrose, suggesting that transfer of anthers from a high to a normal sucrose concentration during culture would ensure that full advantage is taken of a much higher initial survival on the higher concentration (Dunwell and Thurling, 1985). In *Brassica rapa* microspore culture, there was also an increase in embryo yields, as well as in embryo normality by increasing the concentration of sucrose from 10 to 17% followed by a media change to 10% sucrose after 48 h of incubation (Dias and Marto, 2001).

Plant growth regulators (PGRs) are another significant component in plant tissue culture media. They are introduced as the key signaling molecules to control plant growth and development and the transduction pathways of initial response signals to environmental stimuli (Zur *et al*., 2015). After stress-induced microspore embryogenesis, responsive microspores abandon their natural developmental program to follow an *in vitro* embryogenic pathway, leading to *in vitro* embryo formation. Auxin biosynthesis, transport, and action are required for stress-induced microspore embryogenesis. The findings have revealed not only that the induction of auxin biosynthesis gene and intracellular auxin accumulations are associated with the initiation of stress-induced microspore embryogenesis, but also auxin biosynthesis is required for correct *in vitro* embryo development, from initial until advanced stages (Rodríguez-Sanz, 2015; Pérez-Pérez *et al*., 2019). In a highly embryogenic spring rapeseed line, a concentration of 1.01 µM of endogenous auxin (IAA) was detected in microspores subjected to heat treatment in order to induce embryogenesis (1 day at 32 °C). It could be supposed that such IAA concentration is optimal for further embryo development (Dubas *et al*., 2014). In eggplant microspore culture, after the heat stress-dependent embryogenesis induction, a combination of exogenous auxin and cytokinin is required to control the growth and differentiation of the induced microspores (Miyoshi, 1996; Corral-Martínez and Seguí-Simarro, 2012; Hashemi *et al*., 2023). The concerted action of exogeneous auxins and cytokinins can control *in vitro* plant cell division and morphogenesis. These two hormone groups usually act antagonistically but their effects are modulated by plant genome and tissue specificity (Moubayidin *et al.,* 2009).

This study evaluated the effects of different concentrations of gum arabic, sucrose, and plant growth regulators (BAP and NAA) on the androgenic response and the development of globular embryos into later embryonic stages in isolated microspore culture of eggplant.

MATERIALS AND METHODS

Plant material and plant growth conditions

Two eggplant F1 cultivars, Ricarda (agro-TIP, Germany) and Chantale (Seminis, USA), were used as donor plants. The seeds were grown in nursery trays with peat moss and perlite in a 3:1 ratio at 25 °C with a 16-h/8-h light/dark photoperiod cycle for 1.5 months in a controlled plant growth chamber (9000 lux, RH 60-70%,). Irrigation was done every day. After reaching the 4-leaf stage, plants were transferred to a plastic greenhouse (GPS coordinates: 51 °09′ E longitude and 35 °44′ N altitude, 1265 m above sea level) at 25-29 °C/15-19 °C (day/night) with natural light (30000-40000 lux). Plants were irrigated every three days and fertilized once every three weeks with a complete fertilizer (20N-20P-20K). Flower buds from late June to late September were used for the isolated microspore culture.

Isolated microspore culture

Flower buds at the appropriate stages of microspore

development (late uninucleate and early binucleate) were collected and washed for five min with 3-4 drops of liquid dish soap. Then, they were placed in a laminar air flow cabinet and surface-sterilized in 70% ethanol (v/v) for five min followed by 20 min in 1% (w/v) bleach with four drops of Tween 20 (Merck, Darmstadt, Germany). Finally, they were rinsed three times with sterile distilled water. The anthers of sterilized flower buds were excised and blended (with a Waring Blender, MC2-37 to 110 ml, Clarkson Laboratory and Supply Inc., Chula Vista, CA) in 20 ml of pre-cooled sterile deionized water twice for 10 seconds and 5 seconds at the low speed. The blender cup had already been chilled in the fridge. The suspended extract was filtered through a 40 µm nylon mesh sieve and then centrifuged at 113 g for 4 min (at 25 °C), followed by washing three times with refrigerated sterile deionized water. The isolated microspores were suspended in sterile, deionized water at a density of about 200,000 per ml, plated in 6-cm plastic Petri dishes, and incubated at 35 °C in the dark. A counting chamber (Fuchs-Rosenthal, Bright-line, Optik-Labor, Germany) was used to calculate cell densities. The microspores were collected three days after the heat and starvation stress via centrifugation at 113 g for three min and resuspended in a liquid NLN medium (Lichter, 1981) without potato extract (pH 5.9 and sterilized by a 0.22 µm cellulose acetate filter) supplemented with 0.5 mg/l BAP, 0.5 mg/l NAA and 2% sucrose (Corral-Martínez and Seguí-Simarro, 2012). They were then incubated at 25 °C in darkness for one month.

Study of the effects of the cultivar, gum arabic, sucrose, and plant growth regulators (BAP and NAA)

This experiment evaluated the effects of the cultivars (Ricarda and Chantale), different concentrations of gum arabic (0, 1600, 1800, 2000, 2200, 2400, 2600, 2800, and 3000 mg/l), sucrose (1% and 2%), and plant growth regulators, BAP and NAA (both 0.5 and 0.1 mg/l), in liquid NLN medium, on the production of microspore-derived calli or embryo-like structures. The treatments were applied after three days of starvation and the exposure of microspores to a heat shock.

Data collection and statistical analysis

In a factorial experiment with a completely randomized design, the experiment was performed with five replicates of each treatment under the same conditions and at the same time. Each replicate consisted of a plastic Petri dish (60×10 mm). Each Petri dish consisted of 6 ml of culture medium with a density of approximately 200,000 microspores per ml.

Two characteristics including the total number of calli and the number of calli≥1 mm per Petri dish were determined after one month of microspore culture. The data were analyzed using the Wald Chi-Squared test in non-parametric generalized linear models. Pairwise comparisons were conducted using the Kruskal-Wallis test. These methods allowed us to extract meaningful insights from the data without making any assumptions about the underlying distribution or structure of the dataset. Through this comprehensive approach, we were able to identify significant patterns and trends, which helped us draw robust conclusions and make informed decisions. These tests were carried out in SPSS Statistics version 16.0 and SAS version 9.0. All figures for the mean comparison were made by GraphPad Prism 7 software.

RESULTS

The process of microspore embryogenesis was induced after applying the combined stress of starvation and heat. A few days after microspore culture, the sporophyte pathway started by microspores dividing. These divisions were visible by light microscopy. During the first seven days of culture in NLN medium, bi-, three-, five- and multi-celled microspores were observed in both studied cultivars (Figure 1).

The effects of cultivar, gum arabic, sucrose, and plant growth regulators (BAP and NAA)

The way the different components of the medium act together has an impressive impact on how the androgenesis pathway develops. Furthermore, genotype plays a significant role in androgenesis. This experiment studied the interaction effect of cultivars (Ricarda and Chantale) and the concentrations of gum arabic, sucrose, and plant growth regulators (BAP and NAA) on the production of microspore-derived calli. Wald χ^2-test (Table 1) showed that the main effects of studied factors and all their interactions on the total number of calli and the number of calli≥1 mm were highly significant (p<0.01). Therefore, mean comparisons were carried out only for the four-way interaction effects.

One of the most important steps for increasing the efficiency of the doubled haploid technique is to find the genotypes that respond well to androgenesis. Cultivar Ricarda produced a lot more total calli (683.53 per Petri dish) than cultivar Chantale (43.73 per Petri dish) in the control concentrations (2% sucrose, 0.5 mg/l BAP, 0.5 mg/l NAA, without gum arabic). Although the total number of calli in cultivar Chantale was much lower than that in cultivar Ricarda but 75% of them were≥1 mm while that of cultivar Ricarda was

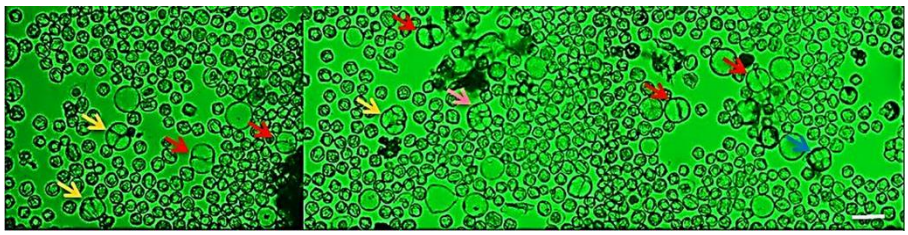

Figure 1. Microspores of eggplant (cultivar Ricarda) divide in the first week after being moved to NLN medium. The stages with two cells (red arrows), three cells (yellow arrows), five cells (blue arrow), and more than five cells (pink arrow) were represented. *Scale bars* indicate 50 µm.

Table 1. Maximum likelihood analysis of variance for the influences of different experimental factors on the microspore androgenesis of *Solanum melongena* L.

Source of variation	df	χ^2	
		Total number of calli/Petri dish	The number of calli≥1mm / Petri dish
C	1	1496013.469**	70420.069**
GA	8	486796.706**	103022.450**
S	1	389996.669**	9.025**
PGRs (BAP+NAA)	1	363855.625**	87.025**
C×GA	8	331910.706**	45012.506**
C×S	1	181755.336**	6873.136**
C×PGRs	1	135528.403**	11685.003**
GA×S	8	513339.306**	107936.550**
GA×PGRs	8	550583.950**	119419.650**
S×PGRs	1	2042286.736**	124062.469**
C×GA×S	8	547687.639**	59240.939**
C×GA×PGRs	8	571592.772**	53585.172**
GA×S×PGRs	8	459508.039**	103371.306**
C×S×PGRs	1	1444380.025**	64026.669**
C×GA×S×PGRs	8	316785.950**	53548.406**

C: Cultivar, GA: Gum Arabic, S: Sucrose, PGRs: Plant Growth Regulators.
**P<0.01

about 10% (Figure 2). Cultivar Ricarda produced the highest total number of calli with combining 2000 mg/l of gum arabic, 2% sucrose, and 0.5 mg/l of BAP and NAA (711.4 per Petri dish), which showed an increase of 1.04-fold compared to the control (683.53 per Petri dish) (Figure 2A). However, in the cultivar Chantale, combining 2600 mg/l gum arabic, 2% sucrose, and 0.5 mg/l BAP and NAA showed the highest total number of calli (230.33 per Petri dish), which was 5.27-fold higher than the control (43.73 per Petri dish). The results also revealed that combining 2600 mg/l gum arabic, 2% sucrose, and 0.5 mg/l BAP and 0.5 mg/l NAA produced the highest mean values (30.3% and 27.3% increases, respectively, compared to the control) for the number of calli≥1 mm in both cultivars (Figure 2B). These results showed that the Ricarda cultivar is a highly responsive genotype to microspore culture.

The direct pathway of microspore embryogenesis starts with the globular stage and ends with the cotyledonary stage in dicotyledonous plants. But this pathway is blocked in the early stage of embryogenesis in some plants, such as eggplant, and replaced by callus structures. After one month of microspore culture in NLN medium, the morphology of the microspore-derived structures was evaluated. The globular structures formed during the first three weeks of microspore culture, but all of them eventually transformed into calli after 30 days. These calli were maintained in the same medium for another three weeks. Only in cultivar Ricarda and with combining 2600 mg/l gum arabic, 2% sucrose, 0.5 mg/l BAP, and 0.5 mg/l NAA, in addition to the globular embryos and callus structures, there were also embryos with an incomplete hypocotyl axis and heart-shaped embryos. But eventually, they all turned into a callus (Figure 3). The mean number of the embryos with an incomplete

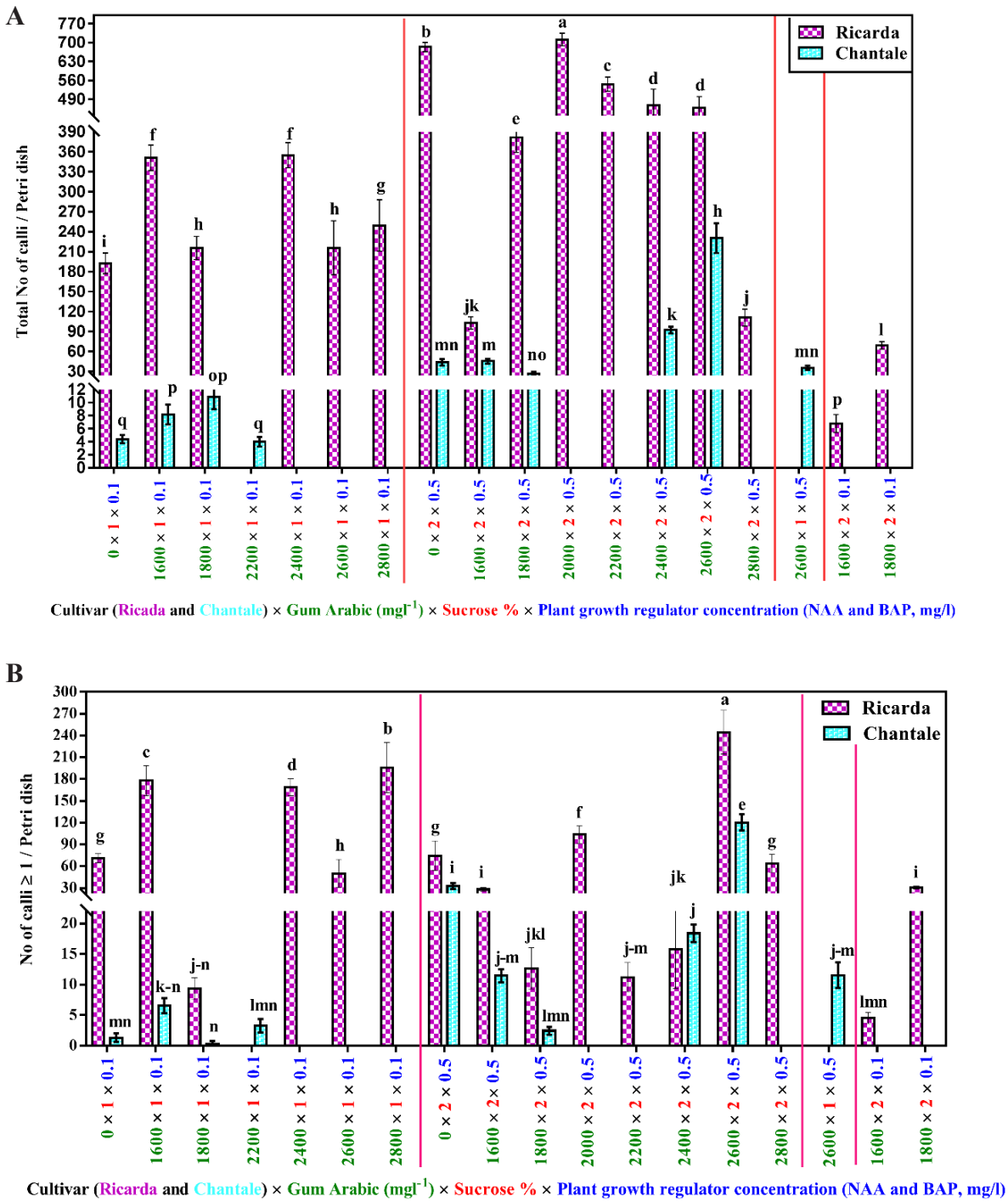

Figure 2. The interaction of cultivar (Ricarda and Chantale), gum arabic (0, 1600, 1800, 2000, 2200, 2400, 2600, 2800, and 3000 mg/l), sucrose (1 and 2%), and plant growth regulators (BAP and NAA: both 0.1 and 0.5 mg/l) on **A:** The total number of calli per Petri dish, and **B:** The number of calli≥1 mm. According to the Kruskal-Wallis test, different letters indicate statistically significant differences.

hypocotyl axis and the heart-shaped embryos was about 54 and 30 per Petri dish, respectively. Heart-shaped embryos and embryos with incomplete hypocotyl axes were observed only in one of the studied treatments, so a statistical analysis was not carried out on them.

DISCUSSION

Anther culture in eggplant has been used to regenerate doubled haploids (Vural and Ari, 2020). However, the isolated microspore culture method is still being looked into because it has more benefits than anther

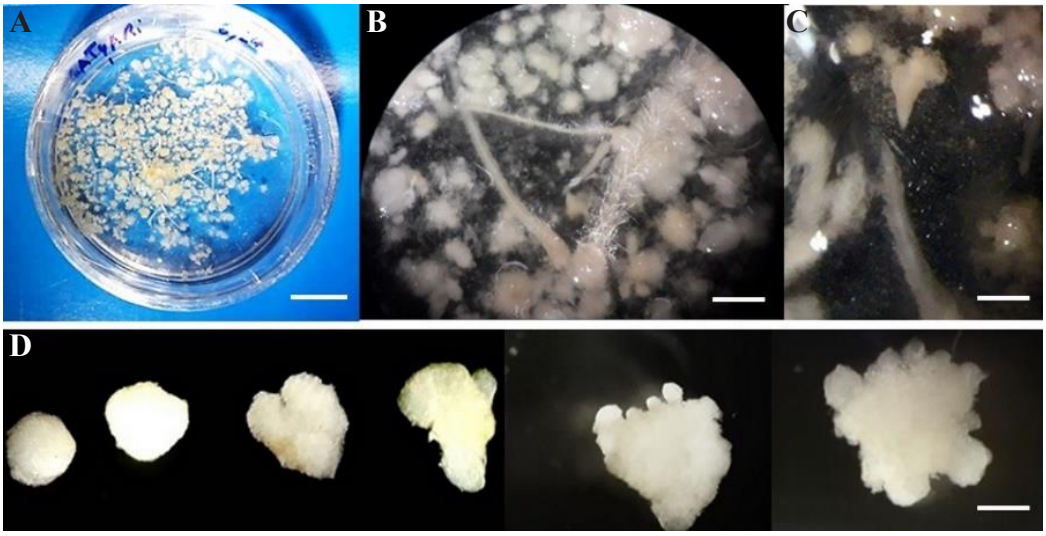

Figure 3. The calli and embryos obtained from microspore culture in cultivar Ricarda with combining 2600 mg/l of gum arabic, 2% sucrose, and 0.5 mg/l both BAP and NAA 45 days after transferring microspores to the liquid NLN medium. **A:** The Petri dish containing calli and embryos. **B and C:** Elongated embryos and the embryos with primary roots, denoted by a yellow and pink arrow, respectively. **D:** Globular embryos (1 and 2), heart-shaped embryo (3), and embryo deformation towards callus formation (4, 5, and 6). Scale bars indicate 1 cm in A, 500 µm in B and C, and 700 µm in D.

culture. By subjecting the microspores to stresses like heat shock and starvation, they can deviate from their gametophytic to their sporophytic pathway. The direct embryogenesis pathway stops at the globular stage in eggplant; the organized embryos become calli (Corral-Martínez and Seguí-Simarro, 2012). So far, there are no reports on eggplant microspore embryogenesis passing through the globular stage, but researchers are still trying to find out how it goes. Indeed, the real transition from the globular stage to the next embryonic stages is the principal bottleneck of this pathway.

In the present study, the effects of cultivar and different concentrations of gum arabic, sucrose, and plant growth regulators (BAP and NAA) were investigated. Finding the highly responsive genotypes to androgenesis is a key step in developing an efficient embryogenesis pathway. Corral Martinez and Seguí-Simarro (2012) introduced the cultivar Bandra as a reference for subsequent analyses due to its high efficiency in producing proliferative structures. Rivas-Sendra *et al*. (2017) reported that the doubled haploid DH36 is morphologically and reproductively similar to the donor hybrid (Bandra), but consistently produced four times more calli (267.36 vs. 65.08). Ozdemir Celik and Onus (2018) showed differences between the three studied cultivars in terms of the number of calli per Petri dish (31.44, 10.22, and 0.0 in cultivars Amadeo, Faslis, and Anamor, respectively). Our results showed that cultivar Ricarda produced the highest total number of calli per Petri dish (683.53). It can be introduced as a highly responsive cultivar for isolated microspore culture in eggplant. This cultivar can be used to develop the embryogenesis pathway.

The medium composition is one of the most important factors for *in vitro* plant growth and development. The carbohydrate is a major component of culture medium because it provides the energy necessary for growth and developmental processes of microspores and also acts as an osmotic regulator. (Cristea *et al*., 2013). Sucrose is often used as a source of carbohydrates and an osmotic agent for the *in vitro* culture of anthers and microspores (Ferrie *et al*., 1995). Microspore survival can also be affected by sucrose concentration (Dunwell and Thurling, 1985). Osmotic pressure is an important factor in the development of structures derived from microspores. Osmotic pressure and kind of sugar had a great influence on androgenesis induction in microspore culture in maize but much less influence on the number of macroscopic structures formed. In media containing sucrose the osmotic pressure rises significantly due to sucrose hydrolysis. In the media containing maltose or mixtures of glucose with fructose, changes in osmotic pressure are much smaller or not significant (Góralski *et al*., 2002).

A high concentration of sucrose was used in the highly responsive plants to androgenesis, including rapeseed (Custers, 2003), tobacco (Touraev *et al*., 1996), and hot pepper (Kim *et al*., 2008). However, 2% of sucrose was applied to recalcitrant plants, like eggplant (Miyoshi, 1996; Corral-Martinez and Segui-

Simarro, 2012; Calabuig-Serna *et al*., 2021).

Plant growth regulators are another important factor in plant tissue culture. Auxins and cytokinins control cell division and morphogenesis. The plant genome and tissues specifically modulate the effects of these hormones (Moubayidin *et al*., 2009). In androgenesis, extensive remodeling of gene expression defines the switch from the gametophytic to the embryogenic pathway. Most of the genes differentially expressed can be ascribed to three main categories: (1) cellular response to the stress; (2) suppression of the gametophytic program (cytoplasmic cleaning), and (3) expression of the embryogenic program (Seguı´-Simarro and Nuez, 2008). Stress treatment acts as a trigger for inducing the sporophytic pathway, preventing the development of fertile pollen (gametophytic pathway) (Touraev *et al*., 1997). Changes in gene expression profiling have been reported in barley (Muñoz-Amatriaín *et al*., 2009) and *Brassica napus.* (Tsuwamoto *et al*., 2007). Changing the level of endogenous phytohormones is one of the important effects of stress. In *Brassica napus*, Dubas *et al*. (2012, 2013) showed that noticeable changes in the level of IBA and IAA caused by stress treatments are important for microspore embryogenesis. Various combinations of auxins and cytokinins have been used in culture media designated for *in vitro* anther culture, whereas in the majority of isolated microspore cultures exogenous plant growth regulators (PGRs) were not required for microspore embryogenesis initiation. In cases where exogenously hormones are needed, the endogenous level of natural phytohormones and its balance with exogenously applied ones can be crucial both for yield and quality of microspore-derived embryos (Zur *et al*., 2015).

AGPs (Serpe and Nothnagel, 1994; Borderies *et al*., 2004; Letarte *et al*., 2006; El. Tantawy *et al*., 2013; Makowska *et al*., 2017; Pourmohammad *et al*., 2021), sucrose (Ferrie *et al*., 1995), and plant growth regulators (Zur *et al*., 2015) have all been shown to be important for some growth and development processes, like embryogenesis, in different plant species. AGPs are a complex, large, and very diverse group of glycoproteins from the hydroxyproline-rich family in the cell wall. AGPs can be important in isolated microspore cultures (Yuan *et al*., 2012). Gum arabic has been known as a source of AGPs (Silva *et al*., 2020). It is the natural secretion of the branch and stem of *Acacia senegal* (a tropical tree) (Makowska *et al*., 2017). *In vitro* use of gum arabic has led to a better green plant regeneration and better embryogenesis in wheat microspore culture (Letarte *et al*., 2006). Gum arabic has been directly involved in the androgenic development of barley microspores. It also increased the cell viability, the mitotic division of microspores, and the formation of multicellular structures (MCSs) compared to the control and significantly improved the quality of the produced embryos (Makowska *et al*., 2017). The use of gum arabic (120 mg/l) alone or with ten ovaries has shown the highest embryogenesis in the microspore culture of bell pepper cultivars (Pourmohammad *et al*., 2021). In the present study, combining 2% sucrose, 0.5 mg/l BAP, 0.5 mg/l NAA, and 2000 mg/l gum arabic produced the highest number of calli per Petri dish. In the microspore culture of eggplant, Corral-Martínez and Seguí-Simarro (2014) found that using 1600 mg/l gum arabic increased the number of calli. They did not test the concentrations between 1600 and 3000 mg/l.

Our results also indicated that when a high concentration of sucrose (2%) is present, a lower concentration of gum arabic can be used. This may be related to the composition of gum arabic. Arabinogalactan polysaccharides make up almost 90 percent of AGPs, and are composed of galactose, arabinose (major sugars), glucuronic acid, glucose and rhamnose (Yuan *et al*., 2012). The results also showed that no callus was formed at the concentration of 3000 mg/l gum arabic. Because of its high osmotic pressure or high levels of toxic or inhibiting compounds, gum arabic's high concentration may be harmful (Corral-Martínez and Seguí-Simarro, 2014).

Even though some treatment combinations increased the number of calli that formed after one month, they did not affect direct embryogenesis or the transition of embryos from the globular stage to the heart stage. However, around 50 days after microspore culture, the embryonic development stages changed from globular to heart-shaped by combining 2600 mg/l gum arabic, 2% sucrose, and 0.5 mg/l BAP and 0.5 mg/l NAA. Based on what was observed, it was clear that some microspores in NLN medium need more time to develop the embryogenesis pathway. To the best of our knowledge, this is the first report on the formation of heart-shaped embryos in eggplant microspore culture.

CONCLUSION

This study showed that heart-shaped embryos can be produced in the microspore culture of eggplant. The development of embryonic structures after the globular stage was affected by a concentration of 2600 mg/L gum arabic. It was also found that cultivar Ricarda is a highly responsive genotype to microspore culture and can produce heart-like embryos. This cultivar can be introduced as a model for improving eggplant

embryogenesis through isolated microspore culture.

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