

Optimization of Somatic Embryogenesis in Industrial Hemp (*C. sativa*)

Dr. Jessica Dobrin¹, Kerri Rapp², Evan Schwan³, Aruhi Jairath⁴, Suhaas Sontyana⁵, Dr. Shobha Rudrabhatla⁶, Dr. Sairam Rudrabhatla³

¹Mercersburg Academy, ²St Theresa Catholic School, ³Central Pennsylvania Research and Teaching Laboratory for Biofuels, ⁴Hershey High School, ⁵Carnegie Mellon University, ⁶Vytal Plant Science Research

Abstract

Cannabis sativa is an industrially important crop with implications in both medical and textile industries, making determining efficient culturing protocols of distinct interest.

This experiment aimed to determine the optimal somatic embryogenesis pathway for *C sativa*. Six different formulations (CIM-1 to CIM-6) were tested, five of which contained the synthetic auxin 2,4-D and one of which contained the synthetic auxin NAA. Two explant types were cultured onto each media and allowed to develop without exposure to light until calluses were observed. Calluses were then sub-cultured onto shoot induction media.

Methods and Materials

Media	CIM-1	CIM-2	CIM-3
	2mg/L 2,4-D	2mg/L 2,4-D + 20mg/L AgNO3	2mg/L 2,4-D + 1mg/L Ascorbic acid
CIM-4	CIM-5	CIM-6	SIM
2mg/L 2,4-D + 20mg/L AgNO3 + 1mg/L Ascorbic acid	0.5mg/L BAP + 0.5mg/L NAA	2mg/L 2,4-D + 0.5mg/L zeatin	0.5mg/L 2,4-D + 2mg/L TDZ

Table 1. Specific formulations for callus induction media (CIM) 1-6 and shoot induction media (SIM)

Calluses induced from young leaves (L) and cotyledons (C) are totipotent, meaning either specific organogenesis or somatic embryogenesis can result. Highlighting this is the spontaneous formation of roots from callus on a sample of CIM-5C seen in Figure 1, right. Those that do not begin organogenesis are sub-cultured onto shoot induction media



Results

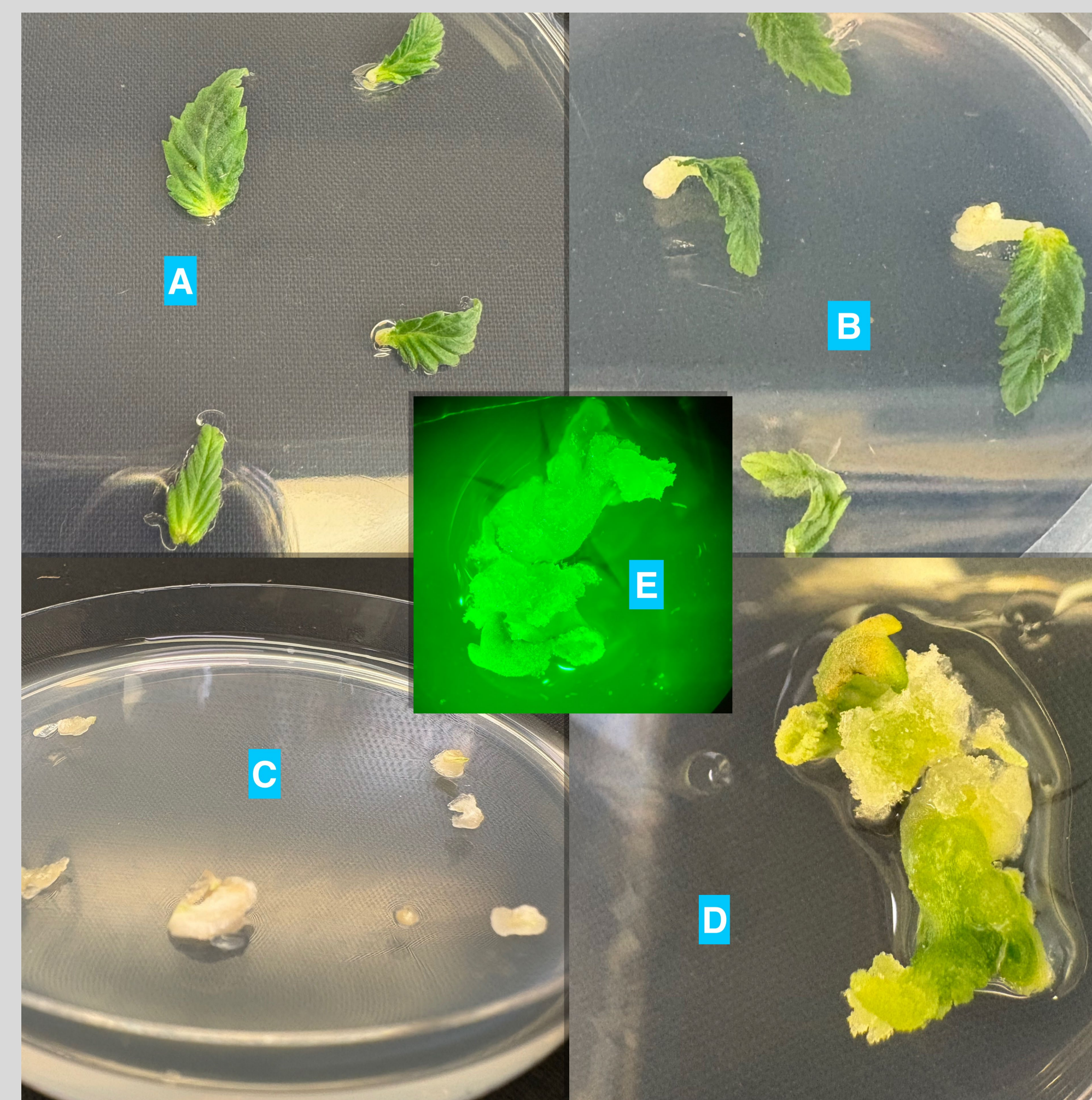


Figure 2. a. Newly cultured young leaf explant b. initial callus formation on young leaf explant c. excised and sub-cultured callus on SIM d. Sub-cultured callus showing signs of initial shooting e. callus from d under fluorescent microscopy

Days to 1st Callus

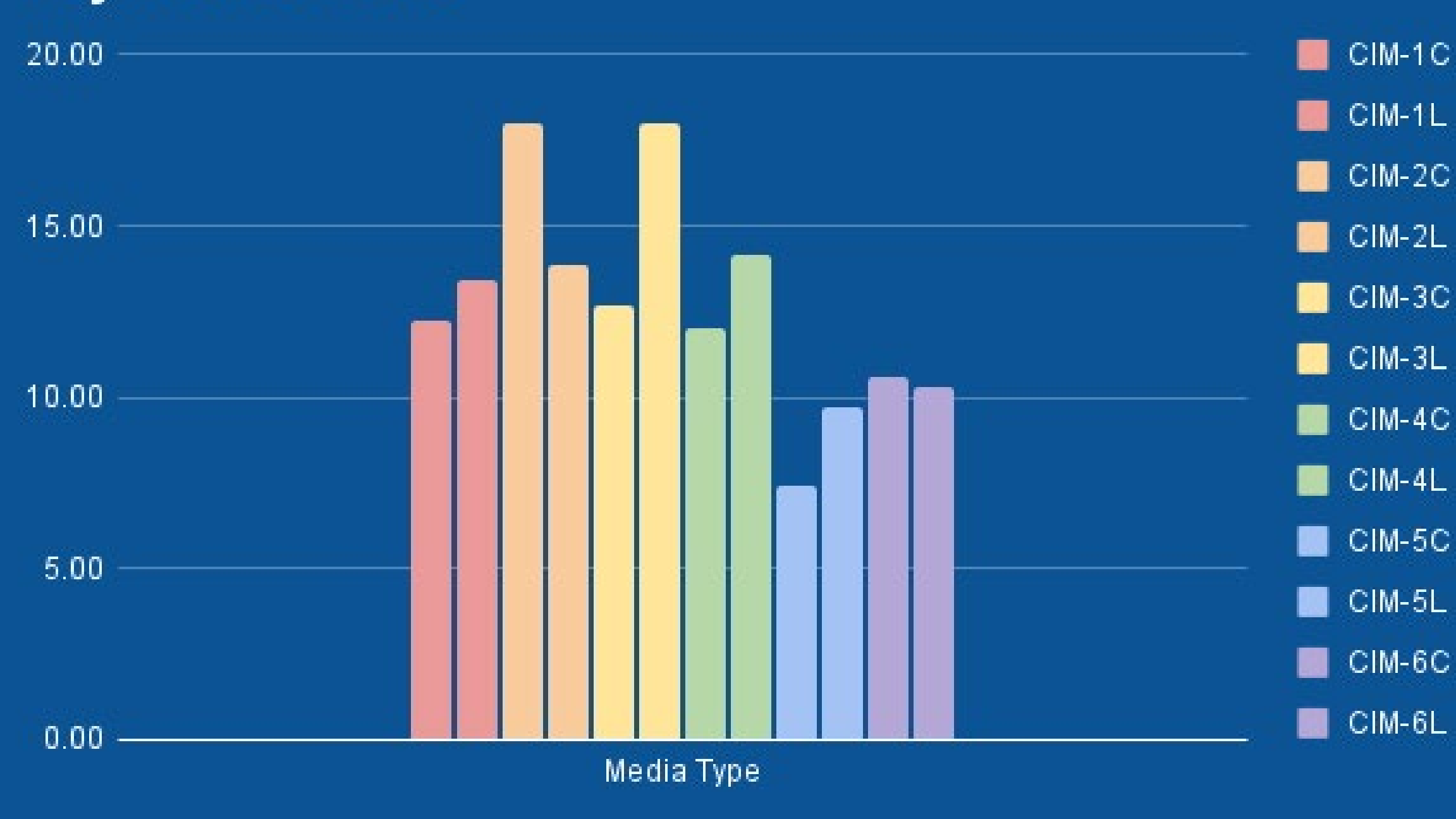


Figure 3. Average days until first mature callus, grouped by media type (CIM-1 to 6) and explant (C or L)

Success rates of explants at first callus

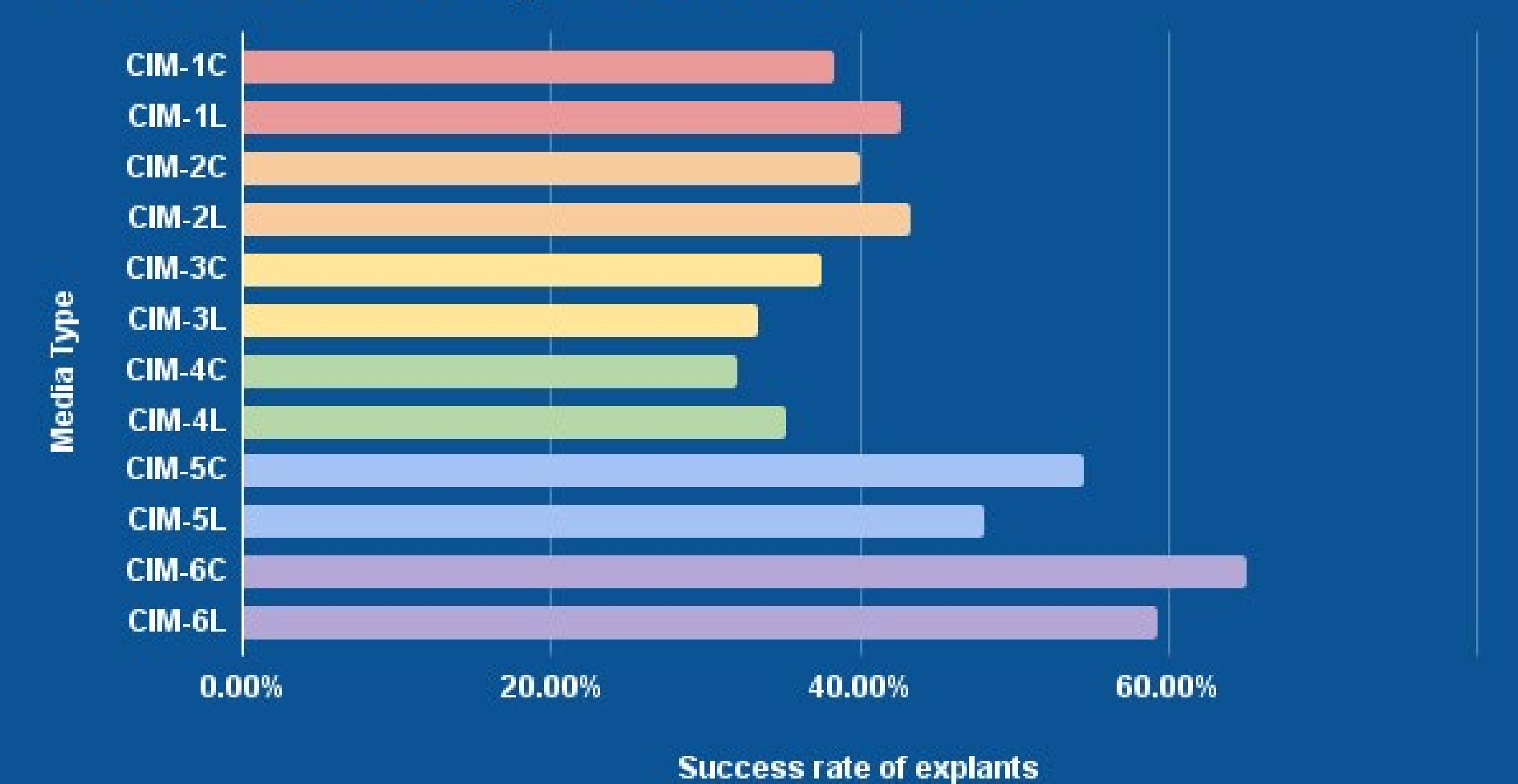


Figure 4. Average success rate of callus formation, by media type and explant

It was determined that CIM-5 was on average the quickest to callus, though CIM-6 had a higher success rate per callus for both explant types



Figure 5. Callus development at (ltr) 5, 14, and 15 days on SIM

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