

Table 1 Composition of solutions and media used for protoplast isolation, transfection and culture, as well as callus culture, somatic embryogenesis and plant regeneration of *C. arabica*

Solution/ medium name	Solution/ medium composition	Application
C, callogenesis medium (1)	half-strength MS (2) salts (Sigma-Aldrich); 40 μ M thiamine HCl, 4.8 μ M pyridoxine HCl, 8.1 μ M nicotinic acid, 13,3 μ M glycine, 0.55 mM myoinositol, 100 mg/L casein hydrolysate, 400 mg/L malt extract (Sigma-Aldrich), 2.26 μ M 2,4-dichlorophenoxyacetic acid (2,4-D), 4.9 μ M Indole-3-butyric acid (IBA), 9.8 μ M 6-(γ , γ -Dimethylallylamino)purine (2iP), 88 mM sucrose, 0.2 % (w/v) Phytigel™, pH 5.6, autoclaved	callus culture initiation
ECP, embryogenic callus production medium (1)	half-strength MS (2) salts, 80 μ M thiamine HCl, 270 μ M glycine, 330 μ M L-cysteine (Sigma-Aldrich), 1.11 mM myoinositol, 150 μ M adenine hemisulfate salt (Sigma-Aldrich), 200 mg/L casein hydrolysate, 800 mg/L malt extract, 4.52 μ M 2,4-D, 17.76 μ M 6-benzylaminopurine (6-BA), 88 mM sucrose, 0.32 % (w/v) Phytigel™, pH 5.6, autoclaved	establishment of embryogenic callus cultures/ protoplast- derived calli proliferation and culture
Lysing enzyme mixture solution	half-strength MS (2) salts without nitrogen, 0.77% (w/v) cellulase RS (Duchefa Biochemie), 0.77% (w/v), macerozyme R-10 (Duchefa Biochemie), 8 mM CaCl ₂ · 2H ₂ O (Duchefa Biochimie), 5 mM MES (Duchefa Biochimie), 0.6 M mannitol (Duchefa Biochimie), pH 5.6, filtered (0.22 μ m membrane; Millex®-GP, Millipore, Merck)	protoplast isolation
W5 solution (3)	2 mM MES, containing 154 mM NaCl, 125 mM CaCl ₂ , 5 mM KCl;), pH 5.7, filtered (0.22 μ m membrane, Millex®-GP, Millipore, Merck)	protoplast purification
CPC, coffee protoplasts culture medium	half-strength MS (2) salts, 80 μ M thiamine HCl, 270 μ M glycine, 330 μ M L-cysteine (Sigma-Aldrich), 16.65 mM myoinositol, 150 μ M adenine hemisulfate salt (Sigma-Aldrich), 200 mg/L casein hydrolysate, 800 mg/L malt extract, 4.52 μ M 2,4-D, 17.76 μ M 6-BA, 88 mM sucrose, 0.55 M glucose, pH 5.6, autoclaved	protoplast culture
Alginate solution	half-strength MS (2) salts, 80 μ M thiamine HCl, 270 μ M glycine, 330 μ M L-cysteine (Sigma-Aldrich), 16.65 mM myoinositol, 150 μ M adenine hemisulfate salt (Sigma), 200 mg/L casein hydrolysate, 800 mg/L malt extract, 4.52 μ M 2,4-D, 17.76 μ M 6-BA, 58 mM sucrose, 0.5 M glucose, 1.6% (w/v) alginic acid sodium, pH 5.6, autoclaved	protoplast embedding
Ca-agar medium	0.6 M mannitol, 50 mM Kill CaCl ₂ · 2H ₂ O, 1.4% (w/v) plant agar (Duchefa Biochimie), pH 5.6, autoclaved	alginate gelation

MMG solution (3)	0.4 M mannitol, 4 mM MES (Duchefa Biochimie), 15 mM MgCl ₂ (Duchefa Biochimie), pH 5.7, filtered (0.22 µm membrane; Millex®-GP, Millipore, Merck)	pre-transfection buffer
PEG4000-Ca ²⁺ solution	0.2 M mannitol (Duchefa Biochimie), 100 mM CaCl ₂ · 2H ₂ O (Duchefa Biochimie), 40% PEG 4000 (Sigma - Aldrich), pH 5.7, filtered (0.22 µm membrane, Millex®-GP, Millipore, Merck)	transfection solution
R, regeneration medium (1)	half-strength MS (2) salts, 40 µM thiamine HCl, 4.8 µM pyridoxine HCl, 8.1 µM nicotinic acid, 82.5 µM L-cysteine, 27 µM glycine, 1.11 mM myoinositol, 400 mg/L casein hydrolysate, 400 mg/L malt extract, 17.76 µM 6-BA, 117 mM sucrose, 0.32 % (w/v) Phytigel™, pH 5.6, autoclaved	plant regeneration
M, maturation medium (1)	semisolid MS (2) medium with full-strength salts, 40 µM thiamine HCl, 0.55 mM myoinositol, 1.3 µM 6-BA, 88 mM sucrose, 0.32 % (w/v) Phytigel™, pH 5.6, autoclaved	plant regeneration

(1) C, ECP, R and M media are described in Dechamp et al. (2015)

(2), MS, Murashige and Skoog (1962)

(3) MMG and W5 media are described in Yoo et al. (2007)