ABSTRACT: Melissa officinalis L. is a perennial aromatic plant used popular in traditional medicine. Its essential oil is recommended for its antimicrobial activity and aqueous extracts exhibit antioxidative properties and anti-inflammatory, anti-tumor and antidiabetic effects. Chitosan is a powerful elicitor that plays a role in plant resistance to stimulate the immunity of plants and has been shown to increase plant growth. This study reports the effect of chitosan with the objective of developing a protocol for the regeneration of lemon balm from callus culture. Shoot explants (hypocotyl, single node and shoot tip) and leaf segments excised from seedlings and cultured on Murashige and Skoog (MS) and Gamborg (B5) media containing different combinations of benzylaminopurine (BAP) (0.0, 1.0, 2.0 or 3.0 mg/L) and naphthaleneacetic acid (NAA) (0.0, 0.5 or 1.0 mg/L). MS medium supplemented with 3.0 mg/L BAP + 1.0 mg/L NAA was the most effective medium for callus production in leaf segments. Calii were placed on regeneration media of MS and B5 and supplemented with different concentrations of chitosan (0.0, 10.0, 20.0 and 30.0 mg/L). According to the results, all chitosan concentrations improved plant regeneration in callus cultures of Melissa. The highest regeneration was obtained from 30.0 mg/L chitosan concentration in single node segments. These findings are the first report of the effects of chitosan on Melissa plant regeneration and shows that chitosan promote regeneration in callus cultures.