

**UNIVERSITI TEKNOLOGI MARA**

**CALLUS INDUCTION ABILITY OF  
ROSELLE (*Hibiscus sabdariffa* L.)  
VARIETIES TERENGGANU AND  
UKMR-2**

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## ABSTRACT

Roselle is known as a multi-purpose plant as it provides many advantages such as for food, colorant and contains highly nutritional values including vitamin C, anthocyanin and hydroxycitric acid (HCA). In Malaysia, roselle has been considered as a relatively new crop and the production is not stable. Among problems faced by the farmers is the need of high quality roselle for better production. Nevertheless, natural and artificial hybridization are difficult to perform due to its cleistogamous nature of reproduction. Therefore, there is an urgent need to develop callus induction protocol for roselle so that it can be used as source materials to improve genetic material and regenerate a high quality roselle. The aims of this study are to establish *in vitro* explant donor for callus induction and to determine the effects of different combinations of 2,4-D + KIN, BAP and BAP + NAA on callus induction using various explants. To achieve these, mature seeds were harvested and surface sterilization was done. Seeds were germinated on ½ strength MS medium and regenerated into plantlets. These plantlets were used as source of explants. Four experiments were conducted. The first experiment, different explants i.e. leaf, hypocotyl (apical, middle, basal) and root were cultured on MS medium with different concentrations of BAP (0.1 mg/L, 2.5mg/L, 5.0 mg/L and 10 mg/L). The second experiment used different combinations of NAA and BAP viz. 1.0 mg/L BAP + 0.1 mg/L NAA, 2.0 mg/L BAP + 0.1 mg/L NAA, 1.0 mg/L BAP + 0.5 mg/L NAA, 2.0 mg/L BAP + 0.5 mg/L NAA and 1.0 mg/L BAP + 2.0 mg/L NAA supplemented with MS medium. The third experiment based on different combinations of 2,4-D and KIN which were 0.0 mg/L 2,4-D + 0.1 mg/L KIN, 0.1 mg/L 2,4-D + 0.0 mg/L KIN, 0.5 mg/L 2,4-D + 0.0 mg/L KIN, 1.0 mg/L 2,4-D + 0.0 mg/L KIN, 0.1 mg/L 2,4-D + 0.1 mg/L KIN, 0.5 mg/L 2,4-D + 0.1 mg/L KIN and 1.0 mg/L 2,4-D + 0.1 mg/L KIN using hypocotyl, leaf and root as explants. The final experiment used *var.* Terengganu and UKMR-2 to test on two different combinations of 2,4-D and kinetin i.e. 0.1 mg/L 2,4-D + 0.1 mg/L KIN and 0.5 mg/L 2,4-D + 0.1 mg/L KIN using hypocotyl, leaf and root as explants. In experiments 1 to 3, *var.* Terengganu was used. Results showed that surface sterilization using 70% ethanol for 30 sec and Clorox mixed with 5 drops of Tween 20 for 5 min resulted in more than 80% germination rate and this technique is recommended for roselle. MS supplemented with 0.1 mg/L BAP gave 100% callus induction from apical, middle-hypocotyl and leaf explants whereas 100% callus survival was observed in all combination of BAP and NAA tested. The best combinations of 2,4-D and KIN for roselle were 0.5 mg/L 2,4-D + 0.0 mg/L KIN, 0.1 mg/L 2,4-D + 0.1 mg/L KIN and 1.0 mg/L 2,4-D + 0.1 mg/L KIN. The range of 2,4-D and KIN obtained was between 0.1 – 2.0 mg/L 2,4-D and 0.1-0.2 mg/L KIN. Besides, different concentrations of plant growth hormones, different explant types, explant age and varieties also effects callus induction of roselle as suggested in other studies. The establishment of callus induction protocol in roselle is important as it can be used in a crop improvement study.

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