ESTABLISHMENT OF CALLUS INDUCTION AND REGENERATION PROTOCOL FOR ANTHERS OF SELECTED BELL PEPPER VARIETIES

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Lack of availability of germplasm is one of the constraints that impede development of local bell pepper varieties. Double haploids generated via anther culture can overcome this problem efficiently with less time consumption. This study was conducted to find out suitable callus induction and regeneration media using two different F₁ hybrids ("Indra" and "King Arthur") of bell pepper. The experiment was carried out in Complete Randomized Design with ten replicates. Anthers were cultured in four different combination of Benzyl adenine (BA) and 2,4-Diclorophenoxy acetic acid (2,4-D) in Murashige and Skoog (MS) medium (T₁ - 1.0 mg/L of 2,4-D + 2.0 mg/L of BA, T₂ - 1.0 mg/L of 2,4-D + 3.0 mg/L of BA, T₂ - 2.0 mg/L of 2.4-D + 2.0 mg/L of BA, $T_4 - 2.0 \text{ mg/L of } 2.4-D + 3.0 \text{ mg/L of BA}$) to select the best callus induction medium. To determine the best regeneration medium, anther derived calli were cultured in MS medium with three different concentrations of 6-Benzyl amino purine (BAP), 2,4-D and Kinetin (T₁ - 0.1 mg/L of Kinetin + 1.0 mg/L of BAP, T₂ - 1.0 mg/L of BA, T₂ - 0.1 mg/L of Kinetin). The results revealed that 2.0 mg/L 2,4-D and 3 mg/L BA combination produced the highest percentage of callus (p<0.05) from anthers "Indra" (75.0%) and "King Arthur" (73.33%). The lowest percentage of callus was recorded in 1.0 mg/L 2, 4-D and 3.0 mg/L BA hormone combination with variety "Indra" (24.36%) and 2.0 mg/L 2.4-D and 2.0 mg/L BA with variety "King Arthur" (33.33%). When callus obtained from all explants were considered together, "King Arthur" recorded the highest number of calli. According to the regeneration experiment, the maximum greening percentage (77.78%) of callus was recorded in 1.0 mg/l of BAP with variety "King Arthur". However, greening of callus was not recorded in "Indra". Callus induction of variety "King Arthur" and "Indra" were best performed in 2.0 mg/L 2,4-D and 3.0 mg/l BA. Regeneration ability of the explants has to be studied further with different levels of cytokines to produce double haploid plants.

Keywords: Anther culture, Bell pepper, Callus induction medium, Regeneration medium