ABSTRACT

In Kenya, rice is a highly important food crop and is considered to be the country's third staple food after maize and wheat. However, there has been a decline in rice production in recent years owing to the changes in climatic conditions, widespread soil degradation and susceptibility of the rice to fungal diseases such as Magnaporthe grisea commonly known as rice blast. To tackle this problem, the country is introducing NERICA (New Rice for Africa) developed by conventional breeding and tissue culture techniques that involved crossing the high yielding Asian rice (Oryza sativa sub species japonica) with the locally adapted African rice (Oryza glaberrima). NERICA requires less water to grow, matures early and produces higher annual yield per unit of land. The objective of this study was to develop an alternative propagation method using tissue culture that might be used for genetic improvement of the rice. The study involved use of dehusked whole rice seeds sterilised using 1.5% sodium hypochlorite. Root and leaf explants were obtained from in vitro germinated plants. The explants were separately inoculated on callus induction medium consisting of N6 macronutrients, B5 micronutrients, N6 vitamins supplemented with 500 mg/l L-glutamine and 0.5 mg/l to 4 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D). Other hormonal combinations used on the N6 basal media for callus induction were 1 mg/l naphthalene acetic acid (NAA) or 1 mg/l 6-benzylaminopurine (BAP) combined with 2 mg/l 2,4-D. All the callus induction experiments were done in both darkness and in light (16 hours light and 8 hours dark) at 26±2° C. The friable calli obtained were subcultured onto to fresh media of the same composition for four weeks before being transferred to Murashige and Skoog (MS) medium with 0.5 mg/l to 3 mg/l BAP combined with 0.5 mg/l to 1 mg/l NAA or 0.2 mg/l IAA for regeneration. MS with 2.5 mg/l kinetin and 0.5 mg/l NAA was tested too. MS basal was used as a control experiment. The regenerated plantlets were planted in sterile soil in pots and covered with a transparent polythene bag to retain humidity Somatic embryogenic calli were produced from whole rice seeds at the optimal concentration of 2 mg/l 2,4-D in darkness between 8-14 days. Calli were obtained from root explants from N6 media supplemented with 2 mg/l 2,4-D and 1 mg/l NAA. No callus was produced from the leaf explants. Callus obtained from root explants gave root regeneration only in all the experiments which were on MS media containing 2.5 mg/l kinetin and 0.5 mg/l NAA, 0.5 mg/l BAP and 0.2 mg/l IAA, 2 mg/l BAP and 1 mg/l NAA and also 3 mg/l BAP and 0.5 mg/l NAA. Embryogenic callus from rice seeds cultured on medium with 2 mg/l 2,4-D gave roots only when transferred to MS medium containing 2.5 mg/l kinetin and 0.5 mg/l NAA. That on medium with 0.5 mg/l BAP and 0.2 mg/l IAA gave shoot buds which did not elongate. Callus on medium with 2 mg/l BAP and 1 mg/l NAA gave plantlets which did not elongate and became necrotic in 2 weeks. Callus subcultured onto to medium supplemented with 3 mg/l BAP and 0.5 mg/l NAA formed roots while that transferred to MS basal medium produced plantlets in six to nine weeks with frequencies of 67% and 77% for NERICA 4 and NERICA 11 respectively. The plantlets transferred to sterile soil survived for 12 days, therefore mature plants were not obtained. This study has demonstrated that NERICA 4 and 11 varieties can be regenerated in vitro from mature embryo explant callus and opens the possibility of future improvement of the crop through genetic engineering.