OPTIMISATION OF REGENERATION AND TRANSFORMATION OF CABBAGE *(BRASSICA OLERACEA VAR CAPITATA)* WITH THE *BACILLUS THURINGIENSIS* (Bt) *CRY 1AC*- GENE.

By

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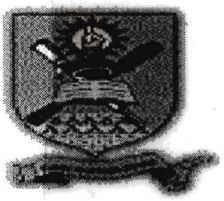
A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE MASTER OF SCIENCE IN APPLIED MICROBIOLOGY AND BIOTECHNOLOGY DEGREE



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February 2012

ABSTRACT

Insecticidal cabbage plants expressing the synthetic *cry*1Ac gene were developed. An efficient regeneration protocol for cabbage was developed by assessing four age groups 7, 14, 21 and 40 day old hypocotyls on five different hormonal combinations in randomized complete block design. Blocking was found to have no effect on regeneration with p values greater than 0.05. Age of the ex-plant was found to have a significant effect on regeneration with a $p<0.01$. The age of choice was 14 day old explants, which had higher mean values for every media for both callus and shoot formation. Different hormonal concentrations in media also had a significant effect on regeneration with a p value less than 0.01. High auxin levels were found to promote callus formation and low auxin levels promoted shoot formation. The addition of 2.4D, a second powerful auxin resulted in more vigorous callusing, production of embryonic cells and multiple shoots. The use of 2.4D enabled lower levels of BAP to be used without significant difference in output produced. Media which contained 5 mg/l BAP, 1 mg/l NAA, and 1mg/l 2.4D and 1mg/l $AgNO_3$ was found to be the best, having the highest mean values for callus formation and producing multiple shoots, a maximum of four shoots per callus. Darkness during culture reduced the time for callus formation but delayed shoot formation and resulted in a higher overall shoot production. Longitudinal dissection to increase cut surface area resulted in the whole explant forming callus but callus initiation was delayed; and shoot and root formation was blocked completely. Antibiotics to be used in eliminating *Agrobacterium* were tested against the bacterial culture and the cabbage and cefotaxime at 150 mg/l was chosen as the antibiotic of choice for eliminating *Agrobacterium*. The gene of interest, the *cry*1Ac gene was confirmed by deploying the polymerase chain reaction (PCR) with *npt*II and universal *cry* primers. Amplicons of size approximately 0.8kb and 1.8kb were obtained for the *npt*II and *cry*1Ac genes respectively. Transformation was successful but regeneration after transformation was minimal, it was delayed and in some cases blocked by transformation and antibiotics used. Only one plant formed shoots and roots after transformation. PCR of transformed plantlets showed successful transformation in one plantlet, giving positive bands of size approximately 860 bp for the *npt*II and 1.8kb confirming the presence of the *cry* 1Ac gene.