Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda

Research Application Summary

Enhancing *Callus* induction and embrogenic cell suspension development in East African highland banana

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Abstract

Improvement of banana using conventional breeding methods is very difficult due to its biology. Genetic transformation is a novel alternative approach for crop improvement. Transformation of EA-AAA bananas has, however, been limited by difficulties in callus induction and embryogenic cell suspension (ECS) development. The existing protocols are not readily responsive. In this study N-phenyl-N'-1,2,3-thidiazol-5-ylurea (TDZ) and N-(2-chloro-4-pyridyl)-N-phenylurea (4-CPPU) that are highly active in regulating morphogenesis in the tissue culture of many plant species have been applied to develop embryogenic callus and ECS culture systems with reliable regeneration efficiency to enhance genetic improvement and propagation of EA-AAA bananas. Preliminary observations indicate that most growth regulator combinations have potential for *Callus* induction.

Key words: 4-CPPU, genetic transformation, morphogenesis, scalps, TDZ

Résumé

L'amélioration de la banane employant des méthodes de reproduction conventionnelle est très difficile à cause de sa biologie. La transformation génétique est une approche alternative originale pour l'amélioration de récolte. La transformation des bananes EA-AAA a été cependant limitée par des difficultés dans l'induction de calus et le développement de la suspension de cellules embryogénique (ECS). Les protocoles existants ne sont pas aisément sensibles. Dans cette étude N-phenyl-N'-1,2,3-thidiazol-5-ylurea (TDZ) et N-(2-chloro-4-pyridyl)-N-phenylurea (4-CPPU) qui sont très actives dans la morphogenèse de régulation dans la culture de tissu de beaucoup d'espèces de plante ont été appliqués pour développer les systèmes embryogéniques de calus et de culture d'ECS avec l'efficacité fiable de régénération pour augmenter l'amélioration et la propagation génétiques des bananes EA-

AAA. Les observations préliminaires indiquent que la plupart des combinaisons de régulateur de croissance ont le pouvoir pour l'induction de calus.

Mots clés: 4-CPPU, transformation génétique, morphogenèse, cuirs chevelus, thidiazol TDZ

Background

Banana, particularly the cooking type East African highland banana (EA-AAA banana) is the mainstay of the majority of people of Uganda. Because of its herbaceous and perennial nature, it is reckoned as an environmentally friendly and sustainable food and income security crop especially in the current event of climate change. Its production however, is constrained by diseases, pests, declining soil fertility and scarcity of clean planting material. Continued and long term banana utilisation inUganda and the greater East African region require banana varieties that can genetically withstand the above biotic and abiotic constraints.

Improvement of banana using conventional breeding methods is very difficult due to its triploid nature, low fertility and limited genetic variability. Genetic transformation is a novel alternative approach for crop improvement. Transformation of EA-AAA banana cultivars has, however, been limited by difficulties in callus induction and embryogenic cell suspension (ECS) development. Embryogenic calli or ECS have been obtained in different banana genome groups and cultivars (Strosse *et al.*, 2003) including the EA-AAA bananas. However, these protocols have not been readily responsive for mass callus induction and ECS development in the EA-AAA bananas to allow routine transformation work. The objective of this study therefore, is to enhance callus induction and embryogenic cell suspension development in EA-AAA banana cultivars through application of highly active urea-type growth regulators.

Literature Summary

Successful application of tissue culture techniques in crop breeding demands callus growth and plant regeneration potential of each crop species or variety to be determined (Khaleda and Al-Forkan, 2006). Plant cell differentiation and morphogenesis *in vitro* is highly dependent on culture medium and conditions. The presence of optimal proportions of growth regulators especially auxins is necessary for successful induction and maintenance of embryogenic cell suspension cultures (Gomez *et al.*, 2000).

N-phenyl-N'-1,2,3-thidiazol-5-ylurea (thidiazuron) (TDZ) and N-(2-chloro-4-pyridyl)-N-phenylurea (4-CPPU), both substituted phenylurea compounds are reported to be highly active in regulating morphogenesis in the tissue culture of many plant species (Victor *et al.,* 2004*;* Haruki *et al.*, 2007). They are known to exhibit a unique property of mimicking both auxin and cytokinin effects on growth and differentiation of cultured explants (Murthy *et al*., 1998).

Study Description

Four cultivars based on the farmers' preference and representing different clone sets namely Mpologoma, Mbwazirume, Nfuuka and Nakabululu are being used in this on-going study. Both shoot tip and immature male flower buds are being used as explants. The shoot tip explants have been first cultured on various media derived by modifying the banana multiplication medium described by Talengera *et al.* (1994) with a combination of TDZ and 4-CPPU at equal concentrations of 5µM, 7µM, 9µM, 11µM and 13µM corresponding to M1, M2, M3, M4 and M5 and, single concentrations at 26µM designated as M6 and M7 respectively to generate scalps. Fifty shoot tips have been used in each treatment and placed in dark growth room in a completely randomized design (CRD).The cultures have undergone monthly sub culturing until scalps were formed. The rate of scalp formation in the various media has been determined by counting the number of shoots and multiple buds in each cycle.

The ideal scalps and immature male buds have been cultured separately on callus induction medium derived by combining 5µM 2,4-D with TDZ and 4-CPPU singly and in combination at 5µM, 10µM, 15µM and 20µM. Fifty explants have been used in each treatment and placed in dark growth room in a completely randomized design (CRD). The percentage of ideal callus formed will be determined as the proportion of scalps or male buds giving ideal callus to the total number of scalps or male buds initiated on callus induction medium. The friable callus obtained will be used to develop ECS lines and consequently regenerate plants. Data will be subjected to ANOVA using Genstat (Release 4. 24DE, 2005). Orthogonal comparisons will be done on number of shoots, multiple buds, percentage ideal callus generated, ECS lines and plants regenerated across cultivars, explant source, subculture cycles and growth regulator combination.

Research Application

Early observations of the male bud cultures show callus starting forming in most of the growth regulator combinations. The expected output of this infant study is to have embryogenic callus and ECS culture systems with reliable regeneration efficiency developed to enhance genetic improvement and propagation of EA-AAA bananas.

Acknowledgement

The study is funded by World Bank and the Government of Uganda through the Millennium Science Initiative (MSI) Program of the Uganda National Council for Science and Technology (UNCST).

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