IN VITRO PROPAGATION OF CASSAVA PLANTS

By

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B.Sc. Agric. Sc. (Veg. Crops), Tanta University, 1999

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ABSTRACT

Neama Abd El-Moneim Abd El-All: *In Vitro* Propagation of Cassava Plants. Unpublished M.Sc. Thesis, Department of Horticulture, Faculty of Agriculture, Ain Shams University, 2013.

Stem nodes of cassava (*Manihot esculenta* Crantz), American cultivar, were treated with different concentrations of clorox at different times (10, 20 and 30 % clorox for 5, 10 and 15 min). The lowest significant contamination and the highest significant survival percentages were recorded with 20% clorox for 15 min. Sterilized and survival nodal explants were cultured on MS medium supplemented with different concentrations of BA and Kin (0.1, 0.3, 0.5 and 1.0 mg L^{-1} from each one alone) incombination with 0.05 mg L^{-1} of NAA. Maximum number of shootlets per explant (5.67) was achieved on MS medium supplemented with 1.0 mg L^{-1} BA + 0.05 mg L^{-1} NAA (proliferation medium). For shootlets multiplication, each shootlet was re-cultured on proliferation medium for two subcultures to investigate the effect of number of subcultures on multiplication rate. Maximum multiplication rate (~ 110 shootlets per nodal explant) was obtained after 75 days of culturing. For shootlets roots formation different concentrations of NAA and IBA (0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1} from each one alone) were used. MS medium supplemented with 2.0 mg L^{-1} IBA achieved the maximum number of roots formation (10.2), root length (14.4 cm) with 100% rooting percentage. *In vitro* derived plantlets were successfully acclimatized on a mixture of peat moss and sand (1:1) which gave the highest percentage of survival transplanting (100%). In addition, callus cultures of cassava were successfully established. Leaf, stem and root explants of one month old *in vitro* growing plantlets of cassava were utilized for callus induction on MS medium supplemented with different concentrations of 2, 4-D (0, 5,

10, 15 and 20 mg L^{-1}). The highest callus production was obtained when leaf explants cultured on MS medium supplemented with 15 mg L^{-1} 2, 4- D where it recorded the maximum values of frequency of callus formation (100%), fresh/dry weights (6.3, 0.581g/jar) and dry matter content (9.2%) with significant differences between treatments. Induced callus was transferred to regeneration medium containing different concentrations of BA, 2iP and TDZ (0, 0.1, 0.5 and 1.0 mg L^{-1} from each one alone). The highest significant regeneration percentage and number of regenerated shootlets (73.3% and 2.2, respectively) were observed on MS medium supplemented with 1.0 mg L^{-1} TDZ. The molecular characterization of the *in vivo* growing plants and the *in vitro* derived plantlets (*in vitro* propagated and regenerated plantlets) was performed. Random Amplified Polymorphic DNA (RAPD) was used to indicate the differences between them using three primers (AM7, AM8 and AM9). Using the primer AM7 gave the highest percentage (100%) of polymorphism. The primer AM7 showed that there were no differences between *in vitro* derived plantlets and the mother plant. Also, protein analysis was performed to detect the differences between mother plants and *in vitro* regenerated plantlets. There was polypeptide band (36 kDa) was over expression found in *in vitro* regenerated plantlet and not detected in *in vivo* plant. So, the differences between in *vivo* plant and *in vitro* regenerated plantlet were not clear. The results demonstrated that there were not observed variations between them. These results confirmed that tissue culture technique was providing safety for *in vitro* propagation of cassava plant.

Key words: *In vitro* propagation, cassava, multiplication, callus cultures, regeneration, molecular characterization.

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Dedication

I dedicate this work to my ***great mother****, my* ***father's soul****, my lovely husband* ***Hassan****, my handsome sons* ***Mahmoud*** *and* ***Abd El-Rahman****.*

CONTENTS

LIST OF TABLES