EFFECT OF SALINITY ON CALLUS FORMATION AND ORGANOGENESIS OF RED KIDNEY BEANS (*PHASEOLUS VULGARIS L.*)

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Abstract

The global warming and its effect on sea level raise is an undisputed fact that has been supported by numerous studies. Countries such as Belize with large area of land just above sea level will face problems due to increased soil salinity. The sea level raise will also affect our fresh water supplies due to back flow in to our rivers. These all will have effect on agriculture since many crops cannot tolerate the increased salinity level. Development of resistant varieties of crops that can withstand moderate level of salinity can help to mitigate the problems of food shortage in the future. Tissue culture or micropropagation can be an effective technique for the *in vitro* development of salt resistant varieties of crops. Initially an experiment was carried out to test the effect of hormones on callus formation of red kidney beans (*Phaseolus vulgaris* L.). Callus tissue was initiated on MS medium supplemented with various hormones (IAA, IBA and 2,4 D) by using explants from epicotyle. Based on the fresh weight produced during the incubation period, 2,4-D was identified as the best hormone for callus growth. After choosing the best hormone for callus generation under *in vitro* conditions, the effect of salinity on callus regeneration and organogenesis of *P. vulgaris* was tested under *in vitro* conditions. Although callus regeneration occurred in all of the salt concentration used in the experiment, the callus regeneration decreased as the concentration of the salt increased. Microscopic evidence of organogenesis was observed as the callus tissue has differentiated in to roots, root hairs and vascular tissues under *in vitro* saline conditions.

Keywords: Red Kidney Beans, Salinity, Callus Formation, Organogenesis

Introduction

Belize has a vast land area that that is marshy and just above sea level. Climate change is no longer a myth and is becoming a fact. The International Panel on Climate Change (IPCC) estimates that the global average sea level will rise between 0.6 and 2 feet (0.18 to 0.59 meters) by end of this century. Rising sea levels threaten the very existence of many small and island nations (IPCC, 2007). With this kind of predictions, a significant land mass may become saline. Even irrigated agriculture land tend become more saline due to application of various fertilizers and evapo-transpiration. It is estimated that about 25% of cultivated land have excess salinity (Shannon 1999).

The Red Kidney Beans (*Phaseolus vulgaris* L.) is a major staple food source in Latin America and particularly in Belize. It has high protein content and plays a significant role in the cultural and economic activities of Belize. In vitro selection on NaCl-containing media seems a promising approach for selecting cell lines which tolerate salt in their nutritional environment. Despite the complex nature of salt tolerance expressed by plant cells, there are reports on the *in vitro* isolation of salt tolerant cell lines and the plants regenerated from such cell lines displaying acquired traits of tolerance at the whole plant level (Croughan et al. 1981, Winicov 1996). Hence grain legumes have a poor regeneration capacity under in vitro conditions (Veltcheva and Svetleva 2005), and in light of the sea level rise and its effect on soil salinity, an experiment was carried out to test the ability of *P. vulgaris* to form callus and undergo organogenesis under *in vitro* saline conditions. The experiment was carried out in an effort to develop a salinity resistant variety of red kidney beans.

Materials and Methods

Seeds of *P.vulgaris* were surface sterilized and germinated under *in vitro* conditions as per standard tissue culture procedure. Explants from epicotyole were used to initiate callus.

The culture media consisted of mineral nutrients defined by Murashige and Skoog (Murashige and Skoog, 1962) and organic substances. The organic substances used were; myo-inosytol (1mg/L), thiamine (1mg/L), nicotinic acid (5mg/L), pyridoxine (0.5mg/L), kinetin (1mg/L), sucrose (30g/L), and agar (8g/L). For the study of effect of hormones the treatment included Indole Acetic Acid (IAA - 2mg/L), Indole Butyric Acid (IBA-2mg/L), and Dichloro-phenoxyacetic acid (2, 4-D-1mg/L). For the study of effect of salinity the treatment included 0.2%, 0.4%, 0.6%, 0.8%, 1.0% of sodium chloride. The PH was adjusted to 5.7. Callus material was weighed

(100 to 150 mg) and placed in the media jars with appropriate hormone or salt concentration and was incubated at 24° C and under white florescent light for 16 hours a day. After four weeks the fresh weight of the callus was weighed and the increase in fresh weight for each treatment was calculated. Some jars from the salinity experiment (0.6 %) were kept aside for up to six weeks and the callus was observed for organogenesis under microscope. Each jar had four calli and there were at least 3 jars for each treatment and the results were expressed as the average weight of all calli for each treatment.

Results and Discussion

The plant hormones used were IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid) and 2, 4-D (Dichlorophenoxyacetic acid). It was evident the observation, that the plant hormone 2, 4, -D showed the most prolific callus formation (Figure 1). The hormone 2, 4-D showed four times more callus growth over IBA and almost two times more growth over IAA (Figure 2).

Figure 1: From left to right: Callus on 2,4-D, IAA, IBA (four weeks after inoculation)

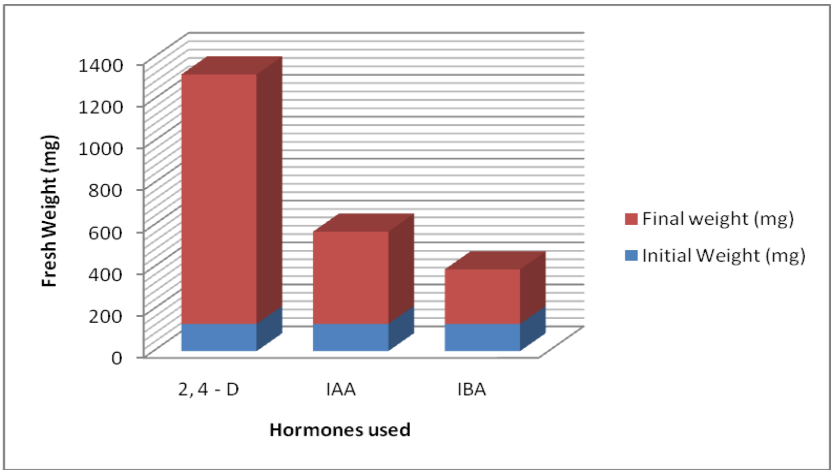

Figure 2: Effect of plant hormones on callus growth

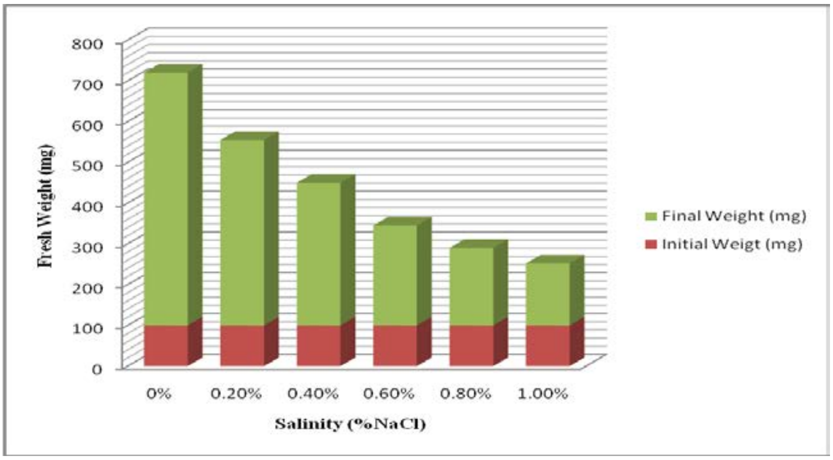

Figure 3: Effect of Salinity on Callus growth of *P.vulgaris*.

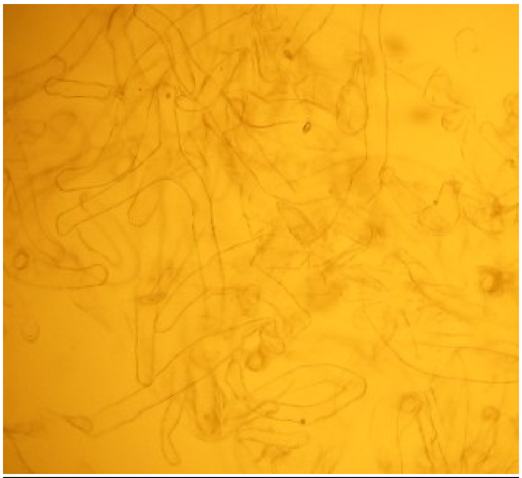

Figure 4: Callus organized to form tissue under *in vitro* saline conditions

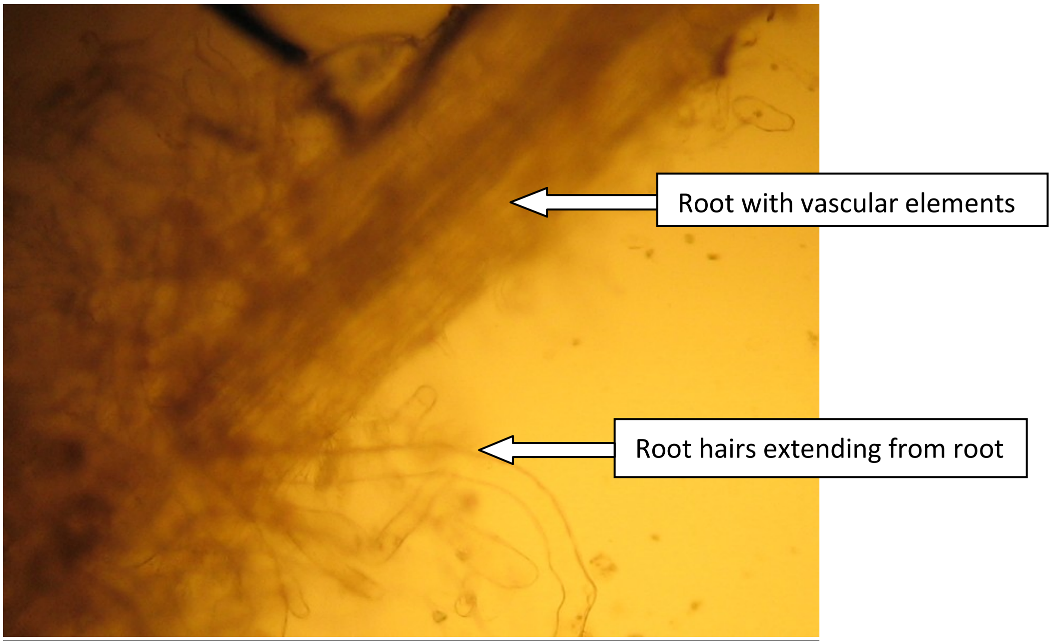

Figure 5: Root and root hair development (organogenesis) under *in vitro* saline conditions.

The callus formation was affected by the salinity of the medium. Although callus growth was observed in all the salt concentrations used in this study, there was a gradual decrease in callus growth as the salinity increased (Figure 3). When Callus from salinity level of 0.6% NaCl was observed for organogenesis, there was clear evidence that cells organized to form tissues (Figure 4) and organs such as root and root hair (Figure 5).

Biotechnology can be of great benefit, especially in the areas of agriculture and medicine. In agriculture, it can be used to improve the yield of crops, increase nutritional qualities of food crops, improve taste, texture or appearance of food, reduce dependence on fertilizers, pesticides and other agrochemicals, and to reduce vulnerability of crops to environmental stresses such as salinity. Micro-propagation is a technique used in biotechnology where a very small piece of tissue (shoot apex, leaf section, or even an individual cell) is excised and placed in sterile culture medium and is used for rapid multiplication of plants. Since conventional breeding programs are not suitable for selection for salt tolerance, organogenesis and/or somatic embryogenesis are effective methods for the isolation and the selection of tolerant lines to generate salt resistant varieties of crops (Vijayan et al. 2003). Our results suggest that 2, 4 D is an ideal hormone that can be used for callus formation. With proper modifications of hormonal concentration and other in vitro growth conditions, callus can be induced for organogenesis and subsequent regeneration of plantlets or somatic embryogenesis.

Conclusion

Phaseolus vulgaris is a staple healthy food source in Latin America and particularly in Belize due to its nutritional value. It is also one of the most cultivated species of leguminous plants in the world. Developing a variety that can withstand saline conditions will be an advantage for countries like Belize where climate change can have drastic effect on salinity of soil in the near future. Work is on the way to generate full plantlets from callus under saline conditions. The work has potential to generate plants that can tolerate moderate saline conditions that may result from sea level raise.

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