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POLYACRYLAMIDE GEL ELECTRROPHORESIS OF SOLUBLE PROTEINS OF SALT AND WATER STRESSED EMBRYO-AXIS OF **Phaseolus vulgaris** L. SEEDS DURING GERMINATION *

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INTRODUCTION

The inhibitory effect of salinity on seed germination has been known for several years.[1-2] However, the mechanisms of action of the salts on this process are not fully understood. Prisco and O'Leary[7] have found that the effects of NaCl on red kidney bean seed germination were primarily osmotic when seeds were in solutions of water potentials of — 8 bars or higher. When the water potential of the substrate was low (— 12 bars) the inhibition of germination was due to both toxic and osmotic effects. The germination process is the result of embryo growth with generation of sufficient force to rupture whatever embryo covers are present, and the growth of the embryo-axis is dependent upon water absorption[9] and protein synthesis.[6] Since one of the known effects of salinity on seed germination is a decrease in water absorption by the seeds it was thought that a correlation between inhibition of germination due to NaCl and the protein synthesizing capacity of the embryo-axis could be found. The protein synthesizing capacity of the embryo-axis was dependent upon water absorption, but this did not explain the differences in germination between NaCl and Carbowax 1540 (osmotic agent) at low water potentials.[8] Another possibility is that the differences in germination when seeds are in NaCl and Carbowax solutions of low water potential are the result not only of the quantitative differences in proteins but also to qualitative differences.

The present experiment deals with the evaluation of soluble proteins from salt (NaCl) and water stressed (Carbowax 1540) embryo-axis of red kidney bean seeds during germination.

MATERIALS AND METHODS

Lots of 10 red kidney bean seeds (W. Atlee Burpee Company, Riverside, California, Lot 233) were placed between folded disposable shop towels (Kimberly-Clark Corporation, Neenah, Wisconsin), covered by a plastic wrap,

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rolled up, and placed upright in 600 ml plastic beakers. Eighty milliliters of solution were used to saturate the towels in each of the treatments. Based on results from previous work, [8] the following water potentials of the substrate were used: 0 (zero) bars (control), —12 bars of NaCl, and —12 bars of Carbowax 1540. The beakers were placed in a dark growth chamber at 25 ± 2°C, relative humidity 95-100%.

After 24, 48, and 72 hours of imbibition, the seed coats were removed and the embryo-axis separated from the cotyledons. The embryo-axis were then homogenized in 0.4 ml of 0.5 M sucrose phosphate buffer pH 7.1, and centrifuged for 20 minutes at 3000 rpm. The supernatant, after assayed for protein [5], was diluted for some of the treatments so all of them had approximately the same protein content. After the preparation of the gels 0.2 ml of the extracts were placed in each tube. The preparation and run of the gels were made according to Davis [3] as modified by Harris and Hall [4]. After staining with 25% Coomassie blue for half an hour the gels were destained in 7% acetic acid + 25% ethanol.

Results and Discussion

At 24 (Fig. 1), 48 (Fig. 2), and 72 (Fig. 3) hours of imbibition the embryo-axis of seeds germinated in water presented the highest number of protein bands. Embryo-axis of seeds germinated in Carbowax either had equal number of bands when compared with embryo-axis of seeds germinated in NaCl (72 hours of imbibition) or the number of bands in the former treatment was higher than in the latter (24 and 48 hours of imbibition). The mobility of the different proteins represented by their *Rf* values was more closely associated in water and Carbowax then when water was compared with NaCl. Prisco and O'Leary [8] have found that seeds in water substrate germinated better than in either Carbowax or NaCl. They have also found that — 12 bars of Carbowax inhibited red kidney bean seed germination less than NaCl solutions of the same water potential. Therefore, the results presented here suggest that the differences in germination between NaCl and Carbowax at low water potentials (-12 bars) are correlated with qualitative differences in embryo-axis proteins. What is not known however, is which of the proteins are responsible for these differences in germination and how they are involved in the inhibition of germination.

Summary

Red kidney bean seeds (*Phaseolus vulgaris* L.) were germinated using sodium chloride (-12 bars) or Carbowax 1540 (-12 bars) solutions as substrates. After 24, 48, and 72 hours of imbibition embryo-axis were excised and their soluble proteins analysed by polyacrylamide gel electrophoresis. Embryo-axis of seeds in water presented the highest number of protein bands at all times studied, while the ones from seeds germinated in Carbowax either had equal or higher number of bands when compared with embryo-axis of seeds germinated in NaCl. The banding pattern was more closely associated in water and Carbowax than when water was compared with NaCl. The results suggest that the differences in germination between NaCl and Carbowax at low water potentials (-12 bars) are correted with qualitative differences in embryo-axis proteins.

Resumo

Sementes de feijão "red kidney" (*Phaseolus vulgaris* L.) foram germinadas em soluções de NaCl ou Carbowax 1540. Após 24, 48 e 72 horas de embebição os eixos embrionários foram extraidos e suas proteinas analisadas por eletroforese em gel de poliacrilamida. Os eixos embrionários de sementes germinadas em água (contrôle)

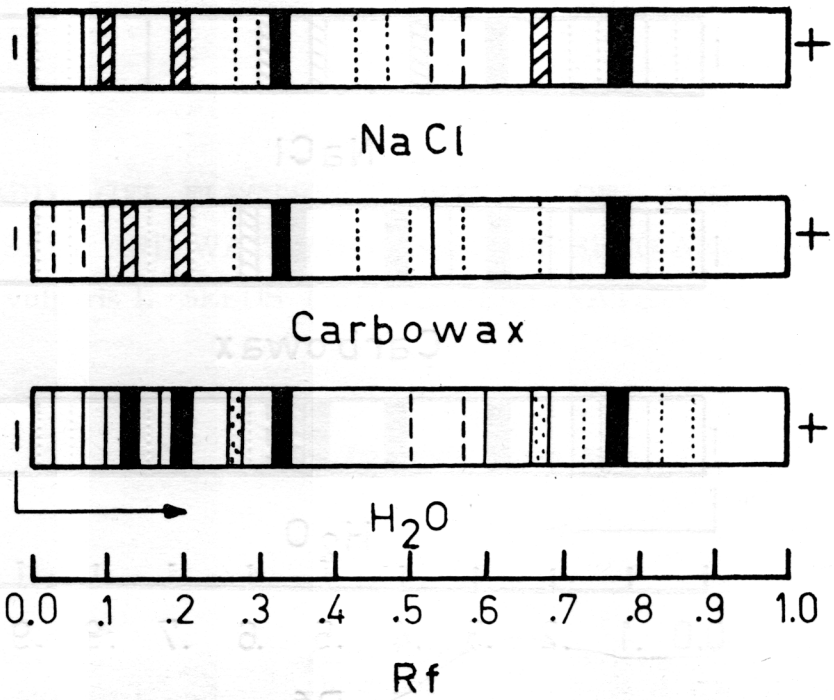

Figure 1. Polyacrylamide gel electrophoresis of soluble proteins of embryo-axis of **Phaseolus vulgaris** L. seeds imbibed in water (0 bars), Carbowax (— 12 bars), and Na Cl (— 12 bars) for 24 hours.

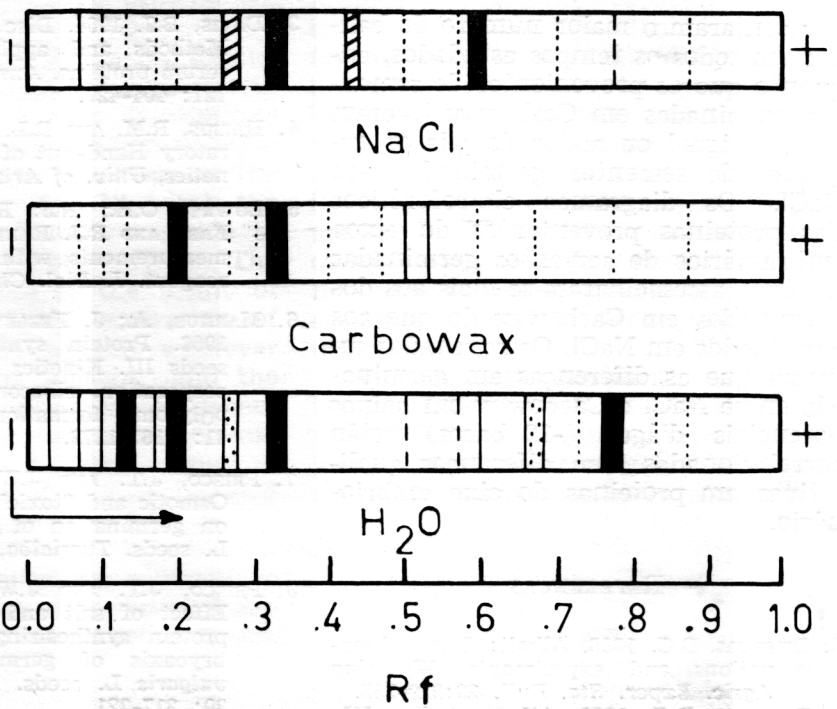

Figure 2. Polyacrylamide gel electrophoresis of soluble proteins of embryo-axis of **Phaseolus vulgaris** L. seeds imbibed in water (0 bars), Carbowax (— 12 bars), and NaCl (— 12 bars) for 48 hours.

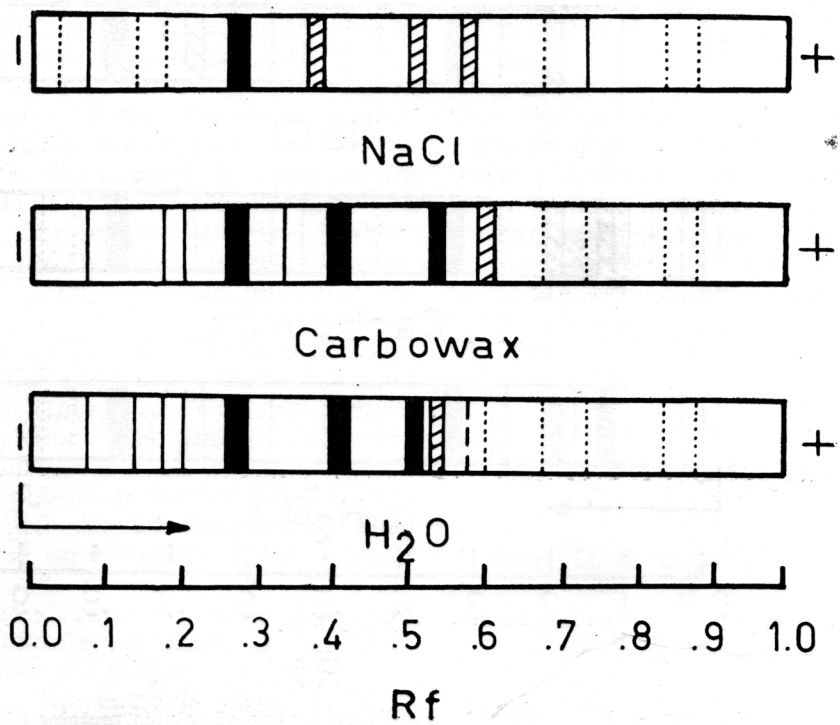

Figure 3. Polyacrylamide gel electrophoresis of soluble proteins of embryo-axis of **Phaseolus vulgaris** L. Seeds imbibed in water (0 bars), Carboawax (— 12 bars), and NaCl (— 12 bars) for 72 hours.

apresentaram o maior número de bandas em todos os tempos estudados, enquanto que os provenientes de sementes germinadas em Carbowax tiveram número igual ou maior do que os extraidos de sementes germinadas em NaCl. Os diagramas eletroforéticos das proteinas provenientes de eixos embrionários de sementes germinadas em água assemelharam-se mais aos dos germinados em Carbowax do que aos germinados em NaCl. Os resultados sugerem que as diferenças em germinação entre NaCl e Carbowax em baixos potenciais d'água (-12 bares) estão correlacionadas com diferenças qualitativas em proteinas do eixo embrionário.

References

1. Buffum, B.C. 1896. Alkali: Some observations and experiments. *Wyoming Agric. Exper. Sta. Bull.* 29:219-253.
2. Buffum, B.C. 1899. Alkali studies III. Germinating experiments with alkali salts. *9th Ann. Rep., Wyoming Agric. Exper. Sta.*, pp. 1-40.
3. Davis, B.J. 1964. Disc electrophoresis II. Methods and application to human serum proteins. *Ann. N. Y. Acad. Sci.*, 121: 404-427.
4. Harris, R.M. and R.L. Hall. 1970. Laboratory Hand-out of Physiological Genetics, *Univ. of Arizona*, 3 p.
5. Lowry, O.H.; N.J. Rosenbrough; A.L. Farr and R.J. Randall. 1951. Protein measurements with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
6. Markus, A.; J. Feeley and T. Volcani. 1966. Protein synthesis in imbibed seeds III. Kinetics of amino acid incorporation, ribosome activation, and polysome formation. *Plant Physiol.*, 41: 1167-1172.
7. Prisco, J.T. and J.W. O'Leary. 1970a. Osmotic and "toxic" effects of salinity on germination of *Phaseolus vulgaris* L. seeds. *Turrialba*, 20: 177-184.
8. Prisco, J.T. and J.W. O'Leary. 1970b. Effect of salt and water stresses on protein synthesizing capacity of embryoaxis of germinating *Phaseolus vulgaris* L. seeds. *Rev. Brasil. Biol.*, 30: 317-321.
9. Walton, D.C. 1966. Germination of *Phaseolus vulgaris*. I. Resumption of axis growth. *Plant Physiol.*, 41: 298-302.