from the Dang Valley lower Siwaliks, 75 miles to the west.

The footprint-bearing level is exposed only locally, as exposure development is directly related to the course of Tinau Khola. Thus it has no significance for regional correlations, and does not help in unravelling Siwalik stratigraphy in the Butwal area.

Biostratigraphic dating of the Tinau Khola section also indicates an early to early middle Siwalik age. The presence, in the middle of the canyon (and thus in the middle of the local sequence), of the suid *Conohyus sindiensis*, a rhizomyid rodent and the primate *Ramapithecus punjabicus*[6] suggests an age there of nine to ten million years[7].

Palaeoenvironment

The palaeoecologic conditions indicated by the footprints and associated trace fossils at loc. N-25 corroborate interpretations of the Nepal Siwaliks based on lithologic and palaeontologic evidence. The impressions were preserved under moist and well-vegetated conditions. The composition of the Siwalik faunas from Nepal (mammals are rare and dominated by large folivorous perissodactyls, while fish and aquatic reptiles are the most abundant vertebrates) suggests abundant moisture and open water. The rocks of the Siwaliks and Nepal are marly, fine-grained, often chemically reduced, and indicate low depositional gradients and a relatively stagnant flow regime.

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1. West, R. M., Munthe, J., Lukacs, J. and Shrestha, T., *Curr. Sci.*, 1975, **44**, 497.
2. West, R. M., Lukacs, J. R., Munthe, J. and Hussain, S. T., *J. Palaeo.*, 1978, **52**, 1015.
3. Johnson, N. M., Opdyke, N. D., Johnson, G. D., Lindsay, E. H. and Tahirkheli, R. A. K., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 1982, **37**, 17.
4. Pilbeam, D., Barry, J., Meyer, G. E., Ibrahim Shah, S. M., Pickford, M. H. L., Bishop, W. W., Thomas, H. and Jacobs, L. L., *Nature (Lodnon)*, 1977, **270**, 684.
5. Colbert, E. H., *Trans. Am. Philos. Soc., n.s.*, 1935, **26**, 401.
6. Munthe, J., West, R. M., Dongol, B., Pant, T. R., Conroy, G., Hutchison, J. H., Kean, W. F. and Munthe, K., *Ramapithecus from Nepal*, Earliest known Asian Hominoid, (in Press).
7. Barry, J., Behrensmeyer, A. K. and Monaghan, M., *Postilla*, 1980, **183**, 19.

CORRELATION OF MEMBRANE RESTING POTENTIAL OF MESOPHYLL CELLS AND DROUGHT RESISTANCE IN WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

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Abstract

Drought susceptible and tolerant varieties of wheat cultivars, exposed to Polyethylene glycol (PEG) induced water stress show marked and systematic differences in the value of the resting potential (E_M) of mesophyll cell membrane. The E_M of susceptible variety invariably depolarized while that of tolerant variety showed hyperpolarization. This correlation is fully substantiated by theoretical considerations of the genesis of resting potential. It is suggested that this technique could provide a reliable index of drought tolerance.

Introduction

In our earlier communication[1] we have put forward the hypothesis that the change in membrane resting potential (E_M) could be used as an index of drought tolerance of crop plants. In contrast to our earlier experiments on coleoptile cells in which the E_M was measured under non-stress condition, in the present study we have measured the E_M of mesophyll cells under the influence of polyethylene glycol (PEG) induced water stress. In order to ensure that during cell's reaction to drought leading to the accumulation of proline[2-4] and malate[5,6] sufficient potassium was available to the cells for its uptake for the purpose of charge balancing[7], we have added 10 mM KCl to the nutrient medium along with PEG.

The uptake of potassium by plants from the medium with additional potassium will lead to a rise in the apoplastic K^+ concentration. This would naturally affect the passive component (E_G) of the membrane potential as given by the Goldman's voltage equation[8,9] leading to membrane depolarization. In accordance with the finding that the cells of drought tolerant varieties have a greater capability of K^+ uptake during water stress[10,11] the E_G of drought tolerant varieties is expected to undergo a greater hyperpolarization than the susceptible. E_M is composed of, apart from E_G, an electrogenic term E_P which depends on cellular metabolism[12-15]. Since metabolism of drought susceptible variety would be impaired to a greater extent under water stress relative to the resistant[2,16,17], the effect of water stress is expected to be a net depolarization in the case of drought susceptible variety, whereas the drought tolerant variety would either show a hyperpolarization or a lesser depolarization as compared to the susceptible. These predictions were fully corroborated by our present study on two wheat varieties, one resistant to drought (C 306) and another susceptible (HD 2204): E_M of the resistant variety showed a net hyperpolarization, while that of the susceptible variety depolarized appreciably.

Materials and Methods

Uniform size seeds of the two varieties C 306 and HD 2204 were germinated as described elsewhere[1] and were transferred after 84 hr to continuous light from white fluorescent tubes giving an average intensity of 2000 lux. After 130 hr of growth the seedlings were flooded with either IX[14] with an additional 10 mM KCl or IX with the additional 10 mM KCl plus 10 and 20% polyethylene glycol – 6000 (osmotic potential – 2 and – 4 bars respectively) solution (pH 5.8) and were allowed to grow for 12 to 16 hr.

Electrophysiological recordings: Healthy seedlings of approximately uniform size were mounted vertically on a perspex frame attached to a perspex chamber (10 × 1.5 × 1.2 cm) with the root system immersed in the test solution. Membrane potential of lower mesophyll cells of the first leaf were measured following the procedure and set up described elsewhere[1]. The reference electrode was placed in the test solution. During recordings the seedlings were illuminated by a microscope illumination lamp with an intensity of about 1000 lux. Separate seedlings were used for each measurement. Results were statistically analysed using students test. Since the E_M values showed only limited degree of dispersion, the number of recordings were limited to 10 in the case of 20% PEG induced stress.

Results and Discussion

In our preliminary study using mesophyll cells we were interested in finding whether the E_M of the mesophyll cells of a given variety differed from that of the coleoptile cells when measured under identical conditions. As given in table 1, the average value of E_M of mesophyll cells of C 306 was – 124 mV compared to – 122 mV recorded for coleoptile cells as reported earlier[1]. The corresponding values for HD 2204 were – 135 mV for both of cells. Thus the E_M of coleoptile cells and mesophyll cells did not show any appreciable difference in both the varieties. The effect of water stress on E_M of the mesophyll cells of the two varieties presented in table 2 shows that the E_M of the resistant variety C 306 hyperpolarized from – 98.9 mV to – 108.9 mV under 10% PEG stress and to – 111.1 mV under 20% PEG stress. The enhanced (negative) value of E_M under 10% PEG can be attributed to a hyperpolarization in E_G due to K^+ uptake by the cells. If the activity of the electrogenic pump of the cells was adversely affected at all, its effect has been masked by the hyperpolarization of E_G. E_M of the susceptible

Table 1

Comparison of the membrane potential (E_M of coleoptile cells and mesophyll cells of wheat varieties

Bathing solution	IX	
Potential difference (mV)		
	C 306	HD 2204
Coleoptile	122 ± 1.57 (28)	135 ± 1.89 (31)
Mesophyll	124.2 ± 2.04 (31)	135 ± 1.81 (32)

Quoted values are mean ± S.E. and number of observations are given in parentheses.

TABLE II

Membrance potential (E_M) of mesophyll cells of wheat varieties under stress and non-stress conditions

	Potential difference (mV)					
	C 306			HD 2204		
Bathing medium:	1X+10 mM KCl	1X+10mM KCl + 10% PEG	1X+10mM KCl + 20% PEG	1X+10 mM KCl	1X+10mM KCl + 10% PEG	1X+10 mM KCl + 20% PEG
	— 91	—111	—114	—110	—110	— 81
	—112	—101	—116	—116	—105	—104
	—108	—115	—102	—118	—106	—101
	—102	—131	—105	—126	— 98	— 84
	— 89	—106	—111	—105	— 98	— 93
	— 98	—121	—120	—115	—121	—112
	— 96	—104	—124	—112	—105	—105
	— 90	—127	—107	—113	—104	— 81
	— 94	—100	— 88	—102	— 77	— 88
	— 82	—112	—124	—121	—109	—100
	—103	—111		—128	—103	— 93
	—107	— 96		—112	— 79	
	—103	—116		—118	— 72	
	—111	—113		—122	— 93	
	—108	—106		—115	— 96	
	—109	—104		—127	—103	
	—116	—102		—115	—110	
	—115	— 97		—112	—100	
	— 91	—111		—106	— 95	
	— 94	— 98		—100	—111	
	— 89	—126		—112	— 92	
	— 92	—128		—102	—109	
	— 96	—103		—106	—109	
	—104	—115		—121	—114	
	— 99	—100		—110	—113	
	— 92	—117		—116	—121	
	—101	—107		—122	—120	
	—102	— 97		—113	— 99	
	— 93	— 93		—114	— 76	
	— 81	— 99		—125	—103	
	— 98				— 99	
					—107	
					—101	
Mean±S.E.	−98.9±1.66	−108.9±1.93[b]	111.1±3.71[c]	−114.5±39[d]	−101.75±2.16[e]	−94.73±31[f]

^{t}a,d=7.26 >99% ^{t}b,e=2.49 >95% ^{t}c,f=2.86>99% ^{t}a,b=3.99 >99% ^{t}d,e=4.91 >99%.

variety on the other hand showed a net depolarization from − 114.5 mV to − 101.8 mV and − 94.7 mV respectively under 10% and 20% PEG induced stress. This can be interpreted as being due to the reduced activity of the electrogenic pump and a lesser uptake or even leaching of K^+ from the cells of the susceptible variety thereby reducing the E_G. Partial plasmolysis of the cells could have taken place in the susceptible variety since wilting was observed in these plants after 20 hr of PEG induced water stress.

Comparison of the E_M values of the susceptible varieties under water stress induced by 10 and 20% PEG show that the magnitude of depolarization increases with increasing degree of stress. In the resistant variety, however, enhanced stress with 20% PEG caused a further hyperpolarization of only − 2 mV which could represent either (i) a marginal further uptake of K^+, or (ii) a higher uptake of K^+ combined with a highly reduced activity of the electrogenic pump in face of the excessive stress. Since wilting was observed in drought-resistant variety also, though much later than in susceptible under 20% PEG stress, we have reason to believe that the metabolic activity of the cells was indeed lowered and hence the second of the above given explanations is, probably more appropriate.

The experimental evidence presented above has fully corroborated our hypothesis and hence we feel justified in our suggestion that E_M can be used as an index of the relative degree of drought resistance of crop plants. Since the experimental technique is rapid and does not involve any damage to the test plant it can be of immense value in breeding crop plants for drought resistance.

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1. Ravindran, A., Ashraf, J. and Amin, M., *Curr. Sci.*, 1981, **50**, 908.
2. Singh, T. N., Aspinall, D. and Paleg, L. G., *Nature New Biol.*, 1972, **236**, 188.
3. Quarrie, S. A., *Ann. Bot.*, 1980, **46**, 383.
4. Singh, T. N., Paleg, L. G. and Aspinall, D., *Aust. J. Biol. Sci.*, 1973, **26**, 65.
5. Cutler, J. M. and Rains, D. W., *Physiol. Plant.*, 1978, **42**, 261.
6. Ergle, D. R. and Eaton, F. M., *Plant Physiol.*, 1949, **24**, 373.
7. Poole, R. J., *Ann. Rev. Plant Physiol.*, 1978, **29**, 437.
8. Goldman, D. E., *J. Gen. Physiol.*, 1949, **24**, 373.
9. Hodgkin, A. L. and Katz, B. J., *J. Physiol. London*, 1949, **108**, 37.
10. Lee-Stadelmann, O. Y. and Stadelmann, E. J., in *Arid land plant resources*, Proc. of the International Arid Land Conference on Plant Resources, Texas Tech. Univ., (eds) J. R. Goodin, and D. K. Northington, International Centre for Arid and Semi-Arid Land Studies, Texas Tech. Univ., Texas, 1979, p. 501.
11. Sinha, S. K., in *Potassium in soils and crops*, (ed.) G. S. Sekhon, Potash Research Institute of India, New Delhi, 1978, p. 223.
12. Higinbotham, N. and Anderson, W. P., *Can. J. Bot.*, 1974, **52**, 1011.
13. Spanswick, R. M., in *Ion Transport in Plants*, (ed.) W. P. Anderson, Academic Press, London, 1973, p. 113.
14. Higinbotham, N., Graves, J. S. and Davis, R. F., *J. Memb. Biol.*, 1970, **3**, 210.
15. Fischer, E., Luttge, U. and Higinbotham, N., *Plant Physiol.*, 1976, **58**, 240.
16. Steward, C. R. and Hanson, A. D., in *Adaptation of plants to water and high temperature stress*, (eds) N. C. Turner and P. J. Kramer, John Wiley, New York, 1980, p. 173.
17. Singh, T. N., Paleg, L. G. and Aspinall, D., *Aust. J. Biol. Sci.*, 1973, **26**, 45.