Epinasty in *Cynodon plectostachyum* R. Pilger induced by Sucrose and its Reversion by Gibberellic Acid and Nitrogen Compounds

Previously evidence has been presented[1,2] to support the view that sucrose is responsible for the prostrate habit of some grasses. Thus it was demonstrated that detached apical pieces of stolons or rhizomes continued to grow horizontally if they were fed with high concentrations of sucrose. Otherwise, these organs would be expected to curve upwards. Further work was succesful in inducing, by mean of sucrose, a downward bending (epinasty) of detached stolons placed in an erect position. In this note, the methods are reported and the results briefly commented.

Apical pieces (15 cm) of 'Star-grass' (*Cynodon plectostachyum*) stolons were excised from plants growing in a field plot. Cut ends of these explants were immersed in 0.02 *M* sucrose (control) or in the test solutions consisting of 0.3 *M* sucrose and/or other substances as indicated in the Table. Each treatment was composed of three 125 ml erlenmeyer flasks containing 50 ml of solution and 4 explants each. Explants were put slightly tilted, about 5° with the plumb line, to secure a transversal geotropic stimulus. All the solutions were prepared with distilled water and renewed every 24 h. Experiments were carried out in a dark culture room (27 ± 1°), except for brief periods of dim light when the solutions were changed. In one experiment explants remained in absolute darkness for 7 days. In this case the test solutions were not renewed and to maintain axenic conditions they were previously autoclaved and the explants surface disinfected with 0.5% sodium hypochlorite for 3 min and rinsed with 0.01% bromine water. Auxin pretreatment consisted of placing the apical pieces upright in 100 ml indol butyric acid (IBA) contained in 200 ml Erlenmeyer flasks under illumination of approximately 300 foot-candle from a fluorescent lamps source (Grolux, Sylvania) at 21 ± 1°. After a 16 h absorption period, explants were transferred to sucrose, sucrose + IBA or IBA solutions alone. At the end of the experimental period (10 days), explants were taken out of the flasks and the deflection angle from a vertical line was measured with a protractor (Figure 1). The data are summarized in the Table.

The results obtained indicate that high sugar supply caused a marked epinasty in the explant internodes (Figure 2). This phenomenon brought the tips to the horizontal direction. Once the tips reached their natural preferred orientation (liminal position), they maintained it throughout the experimental time. No changes in res-

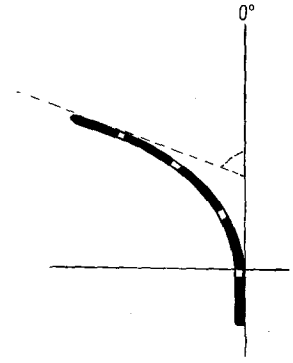

Fig. 1. Schematic representation of the angle used for expressing the curvatures.

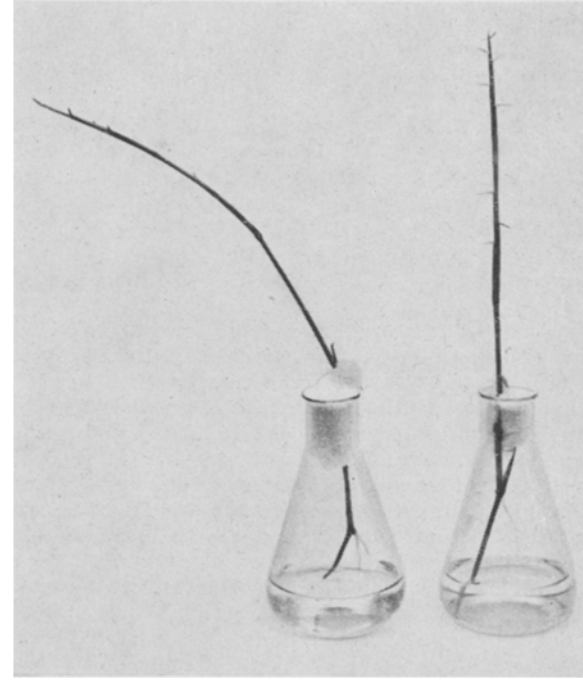

Fig. 2. Epinastic response induced by 0.3 *M* sucrose in *Cynodon plectostachyum*. Control in 0.02 *M* sucrose on right. Explants grown 5 days in the solutions.

Effect of sucrose on the epinasty of *Cynodon plectostachyum* stems in presence and absence of nitrogen compounds, GA_3 and IBA

Treatment	Angle of deflection from a vertical line
0.02 *M* Sucrose (control)	0
0.30 *M* Sucrose	86 ± 3
0.02 *M* Sucrose (absolute darkness)	0
0.30 *M* Sucrose (absolute darkness)	79 ± 2
0.20 *M* NH_4NO_3 + 0.30 *M* sucrose	0
0.20 *M* Urea + 0.30 *M* sucrose	0
10^{-4} *M* GA_3 + 0.30 *M* sucrose	0
10^{-4} *M* GA_3 + 0.02 *M* sucrose	0
5×10^{-5} *M* IBA + 0.02 *M* sucrose	0
12.5×10^{-5} *M* IBA + 0.02 *M* sucrose	0
25.0×10^{-5} *M* IBA + 0.02 *M* sucrose	0
5×10^{-5} *M* IBA + 0.30 *M* sucrose	90 ± 3
12.5×10^{-5} *M* IBA + 0.30 *M* sucrose	81 ± 1
25×10^{-5} *M* IBA + 0.30 *M* sucrose	84 ± 0.8
12.5×10^{-5} *M* IBA[a] + 0.02 *M* sucrose	0
12.5×10^{-5} *M* IBA[a] + 0.30 *M* sucrose	80 ± 2
12.5×10^{-5} *M* IBA[a] + 0.02 *M* sucrose + 12.5×10^{-5} *M* IBA	0
12.5×10^{-5} *M* IBA[a] + 0.30 *M* sucrose + 12.5×10^{-5} *M* IBA	81 ± 2

Average of 12 explants. [a] 16 h pretreatment.

[1] E. R. Montaldi, Experientia *25*, 91 (1969).
[2] E. R. Montaldi, Revta Invest. agric. Buenos Aires, Serie 2, *7*, 67 (1970).

ponses ocurred with IBA given either simultaneously with sucrose or as 16 h pretreatment. Likewise, IBA alone did not elicit any differential growth. On the other hand, bending of the internodes caused by $0.3M$ sucrose was nil in the presence of $0.2M$ ammonium nitrate, $0.2M$ urea or $10^{-4}M$ gibberellic acid (GA_3).

Considering these results they seem to corroborate the hypothesis that the balance sucrose/gibberellin is responsible for the differentiation of diageotropic stems in *Cynodon sp.* However, for this to occur nitrogen there must not be above a certain level. The ratio sucrose/nitrogen as determining whether one stem bud would become a stolon or a tiller is a new factor in the geotropic process, albeit old in other aspects of plant physiology. A recent article[3] points out that the growth direction of *Agropyron repens* rhizomes depends upon nitrogen supply. This finding supports the conclusion that nitrogen also plays a rôle in the plagiotropism of grasses.

Admitting a priori the futility of interpreting the mode of action of these substances, on the ground that the geotropic mechanism in plants is far from being elucidated, a brief speculation may be of value for future work. The hypothesis previously stated [1,2] that in presence of high concentration of sucrose an antihormone would be synthetized on the low side of the stem, counteracting the effect of some growth factor, is still valid. Nitrogen could divert the metabolic sucrose pathway to another process and consequently shift the balance towards the growth promotor.

According to the hormonal theory, the difference in growth rate between the two sides of the curving stem is causally correlated with a differential distribution of auxin. However, considering the inactivity of the auxin in this and other experiments[1,2], it is easier to accept the proposed idea that an anti-hormone is present in the low side of the stems of *Cynodon sp.* The following facts further support this hypothesis: a) Sucrose induces epinasty in the presence or absence of saturating amounts of exogenous auxin. b) Sucrose keeps stolons and rhizomes growing horizontally under similar condition. c) Proofs have been reported[2] that auxins are taken up and distributed to the whole stem piece. d) Auxin destruction is excluded on the basis of its chemical nature. It is unlikely, then, that IBA were not present during the differential growth leading to the epinasty of the internodes. In this case, it is also very difficult to conceive an uneven and subtle distribution of the exogenous auxin in the stems, considering the high concentration utilized and the small differences required to elicit an unequal growth rate on both sides of the internodes.

Summing up, in the plagiotropism phenomenon of *Cynodon sp.*, only the presence of geosensors in the cells can be considered as an obvious fact. Their behaviour depends on the gravitational force and non-specific chemical substances like sucrose and nitrogen compounds, acting probably through an unknown hormone-antihormone system, in which the gibberellins are involved[4].

Zusammenfassung. Nachweis, dass Saccharose in Ausläufern von *Cynodon plectostachyum* Epinastie verursacht, was durch Gibberellinsäure und stickstoffhaltige Verbindungen antagonisiert wird. Indol-3-Buttersäure veränderte die Wirkung des Zuckers nicht.

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8 January 1973.

[3] G. I. MCINTYRE, Can. J. Bot. *50*, 393 (1972).

[4] Sucrose was obtained from BDH, Laboratory Chemical Company, England. Gibberellic acid and indol-3-butyric acid from E. Merck, A. G. Darmstadt, Germany. Ammonium nitrate, potassium nitrate and urea were pro-analysis quality.

STUDIORUM PROGRESSUS

Inhibition of Angiotensin-Converting Enzyme by Analogs of Peptides from *Bothrops jararaca* Venom

The product of the reaction catalyzed by the kidney enzyme renin is a biologically inactive decapeptide, angiotensin I[1]. Further cleavage of this decapeptide to form the vasopressor octapeptide angiotensin II is achieved by action of angiotensin-converting enzyme, a carboxypeptidyl dipeptide hydrolase found in blood and most other tissues[2-8]. This 'converting enzyme' is not specific in its action on angiotensin I, but cleaves dipeptide residues from the C-terminal end of many peptides, including bradykinin[9-14].

The probable physiological importance of angiotensin-converting enzyme, both for production of angiotensin II and for destruction of bradykinin, has prompted us to search for inhibitors of its action. Although many peptides and some nonpeptidic compounds are moderately potent inhibitors of angiotensin-converting enzyme[2,10-12,15-17], the most potent and specific inhibitors by far are several structurally similar peptides that have been isolated from the venoms of the South American pit viper *Bothrops jararaca*[18-21] and the Japanese pit viper *Agkistrodon halys blomhoffii*[22-25]. These venom peptides, the structures of which are shown in Table I, have been demonstrated to be potent inhibitors of angiotensin-converting enzyme in vitro[6-8,10-12,14,26-33] and in vivo[27-29,34-40]; they are competitive with substrates such as angiotensin I and bradykinin[8,26,33].

With the hope of obtaining a better understanding of the structural requirements for inhibition of angiotensin-converting enzyme by venom peptides, we have prepared and tested a large number of analogs of the peptides from *Bothrops jararaca* venom. The structures of these analogs (Table II) are all related to those of the naturally occurring pentapeptide (**1**), nonapeptide (**31**), or decapeptide (**46**); all peptides are aligned to permit direct comparison of their C-terminal amino acid residues. Most of the analogs of the pentapeptide (**2–28**) contain the more stable amino acid phenylalanine, rather than tryptophan, but this substitution does not appear to affect inhibitory activity (cf. peptides **1** and **2**). Peptides were synthesized by solid-phase methods, or in solution by fragment-condensation methods[41,42]. Their inhibitory potencies were tested against activities of crude or homogenous preparations of angiotensin-converting enzyme of rabbit lung, assayed spectrophotometrically or spectrofluorometrically[8,9,26].

Although angiotensin-converting enzyme cleaves dipeptide residues from its substrates, it is an exopeptida-