

different combinations and also the absence of any environmental effect on the frequency of pollen fertility, ruled out cytoplasmic-genetic factor while the observations suggested genic male sterility.

The segregation pattern of the different progenies (table 1) showed that the MF clone 584 was homozygous for MF because of the absence of any MS plant in the OP progeny. However, another MF clone OP-4 was apparently heterozygous because one MS plant appeared in the OP progeny. Besides, the occurrence of the MS plants in the OP progeny of MS clone 539 in the F_1 progeny itself clearly established the presence of heterozygous MF genotypes around the clone in the germplasm. When the MS clone 539 was crossed with the MF clone 613, the occurrence of 23 MS: 24 MF plants in the F_1 progeny suggested that it was only a back-cross ratio of 1:1, wherein the MS clone 539 was homozygous recessive (ms ms) while the MF clone 613 was heterozygous (MS ms), and the male fertility was dominant over male sterility.

Genetics of male sterility controlled by monofactorial recessive inheritance has been reported in more than 100 cultivated plants⁶. It is concluded that the male sterility in the clone 539 is controlled by single recessive gene pair, which is being reported for the first time in cassava.

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1. Jos, J. S. and Nair, S. G., *Cytologia*, 1979, 44, 813.
2. Jos, J. S., Vijaya Bai, K. and Nair, R. B., *Cytologia*, 1983. (in press).
3. Magoon, M. L., Jos, J. S. and Vasudevan, K. N., *Nucleus*, 1968, 11, 1.
4. Jos, J. S., Magoon, M. L., Sadasivaiah, R. S. and Appan, S. G., *Indian J. Horticult.*, 1966, 23, 177.
5. Jos, J. S. and Vijaya Bai, K., *Curr. Sci.*, 1981, 50, 1035.
6. Gottschalk, W. and Kaul, M. L. H., *Nucleus*, 1974, 17, 133.

DIFFUSIVITY OF PROLINE AND ITS SIGNIFICANCE

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MANY solutes are known to accumulate in leaf tissue under moisture stress conditions and proline is one such. Recently, the solubility of proline and its ability to co-exist with other solutes and its biological significance have been shown¹. Proline has certain other unusual properties such as prevention of denaturation of enzymes by heat^{2,3}, micelle formation in solution⁴ and increasing the solubility of proteins⁵.

Under moisture stress conditions, proline has been shown to accumulate essentially in the cytoplasm⁶. However, accumulation in the vacuole is not ruled out. The concentration of proline in the leaves of *Triglochin maritima* expressed on the basis of cell water content may exceed 200 mM. Moreover, if it is assumed that this entire accumulated proline is confined to the cytoplasm, which occupies around 10% of the cell volume, the calculated osmotic potential for this compartment would be -5.6 MPa. This value is very much less than the osmolarity of the cell sap, -2.9 MPa⁷. Hence, some of the proline must be located in the vacuole also. If accumulation in the vacuole can take preferentially, then diffusion from cytoplasm to the vacuole across the tonoplast membrane must be occurring at a fast rate. Perhaps, this compartmentation may be the reason for the lack of a feedback inhibition in proline accumulation under stress.

Experiments were conducted to study the rate of diffusivity of proline and other solutes in plant tissue.

Experiment No. 1: Cylindrical pieces of potato, 2 cm in diameter and 1.5 cm in height were cut. The thickness of the sides and the bottom of the potato cup was 5 mm in each case. Such potato cups were placed in distilled water for 16 hr to remove the easily diffusible endogenous solutes present within the tissue. Each of the test solutions (0.4 ml), proline, glycine, glucose and potassium chloride (0.1 M) and distilled water were taken in the cups and were placed in petri plates with 5 ml of distilled water for 2 hr. Three such osmometers were kept in a single petri plate and three replications per treatment were taken. At the end of the two-hour period, the different solutes in the ambient medium were estimated and used as a measure of differential diffusivity.

Experiment No. 2: In a second experiment, agar blocks of $4 \times 4 \times 4$ mm were equilibrated overnight in 1 M solutions of proline, glycine, glutamic acid, KCl and mannitol (Donor blocks). They were then placed on the morphological apex of 3 mm long cowpea hypocotyl sections. The other end of the hypocotyl sections were dipped into 4 ml of distilled water in a petri plate. The entire system was placed in a humid chamber. The solutes from the donor agar blocks were allowed to move into the water through the hypocotyl sections for 20 min. To study the movement of each solute, 30 such agar blocks were used. At the end of the experiment, the amount of the respective solutes in the water in the petri plates was determined. The donor blocks of each solute were then transferred to 2 ml of distilled water and the residual solute concentration was determined. To measure the initial amounts of solutes present in the agar blocks, 20 blocks were immersed in 2 ml of water at the start of the experiment and the quantity estimated.

In this experiment, standard methods were used for estimation of various solutes. For proline the acid ninhydrin method⁸, for glycine the ninhydrin method⁹, for glucose, the Nelson's chromogenic method¹⁰, flame photometry for potassium and for mannitol, the sodium metaperiodate oxidation method¹¹, were used respectively.

When the diffusivity of the different solutes was

Table 1 Diffusivity of different solutes across a potato osmometer.

Solute	Diffusivity (mmol cm ⁻² h ⁻¹)
Proline	2.2
Glycine	1.4
KCl	0.3
Glucose	0.15

calculated as mmol of solute diffusing out per unit area of tissue per hour, it was found that proline had the highest diffusivity rate (2.2 mmol cm⁻² hr⁻¹) as compared to glycine which was 1.4 mmol cm⁻² hr⁻¹. KCl and glucose had relatively very low rates of 0.3 and 0.15 mmol cm⁻² hr⁻¹ in the potato cup experiment (table 1).

Table 2 gives the amounts of the different solutes in the donor agar blocks at the beginning and at the end of the experiment and also the diffusivity of those solutes through cowpea hypocotyl sections. This experiment reconfirmed the fact that proline exhibits a high diffusivity rate compared to other solutes such as glycine, glutamic acid, potassium chloride and mannitol (table 2).

The observed higher diffusivity of proline in comparison with some of the other commonly occurring solutes in plant tissues, is another unusual property of proline. The biological significance of this could be due to proline diffusion as and when it accumulates across the membrane and accumulation in organelles and thus escaping from oxidation in the cytoplasm. At the same time, by this compartmentation, it could serve as an osmoregulatory solute.

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1. Krishna Sastry, K. S., Udayakumar, M., Devendra, R. and Mekhri, A. A., *Curr. Sci.*, 1982, 51, 485.
2. Pollard, A. and Wyn-Jones, R. G., *Planta*, 1979, 144, 291.
3. Faleg, L. G., Douglas, T. J., van Daal, A. and Keech, D. B., *Aust. J. Plant. Physiol.*, 1981, 63, 17.
4. Schobert, B., *J. Theor. Biol.*, 1977, 68, 17.
5. Schobert, B. and Tschesche, H., *Biochem. Biophys. Acta*, 1978, 541, 270.
6. Goring, von H., Dreier, W. and Heinke, F., *Biol. Rundsch*, 1977, 15, 377.

Table 2 Amounts of the different solutes in the Donor Agar blocks at the beginning and end of the experiment and their diffusivity.

Solute	Diffusivity (mmol cm ⁻² hr ⁻¹)	Initial amount of solute in donor agar block (μg)	Final amount of solute in donor agar block (μg)
Proline	8.8	32,775	8633
Glycine	0.14	19,000	17,550
Glutamic acid	0.004	12,300	9,700
KCl	0.57	18,450	14,800
Mannitol	1.13	12,600	8,400

7. Jefferies, R. L., In: *Genetic engineering of osmoregulation* (eds) D. W. Rains, R. C. Valentine and A. Hollaender, Plenum Press, 1980, p. 381.
8. Bates, L. S., Waldren, R. P. and Teare, I. D., *Plant Soil*, 1973, 39, 205.
9. Spies, J. R., *Methods enzymol.*, 1957, 3, 468.
10. Nelson, N., *J. Biol. Chem.*, 1944, 153, 375.
11. Lewis, D. H. and Smith, D. C., *New Phytol.*, 1967, 66, 185.

ORDERING OF SHOOTS WITH SPECIAL REFERENCE TO *EXBUCKLANDIA POPULNEA*

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ORDERING of branches plays a very important role in determining the tree architecture. Mainly we talk about two types of ordering—botanical¹ or developmental ordering and Strahler's² ordering. In the latter, the terminal branches are of order 1, and two such branches meet to form an order 2 branch and so on down to the trunk. The union of an order i and order j branch is assigned order $i + 1$ when $i = j$ or order $\max(i, j)$ when $i \neq j$. In botanical ordering the leader is said to be of order 1, the primary branches emerging from the leader as of order 2 and so on. This paper attempts to move a step further by ordering the individual shoots, instead of branch as a whole. The method of ordering modifies the earlier one, in its ability to identify each shoot individually with respect to its position in the canopy, its age or developmental stage. Thus, in addition to describing the tree architecture in greater detail, it also helps in recording the various characters, (quantitative or qualitative) of individual shoots. It can be used very efficiently for demographical studies of shoots or even leaves. The method has been developed with special reference to *Exbucklandia populnea* (Hamamelidaceae). Possibilities and limitations of using this method to other tree species are also discussed.

E. populnea is a medium-sized to tall tree. Our observations are based mainly on 4 to 5-year old

saplings. So far, in this species we have observed branches upto order 4 (taking leader as of order 1). On these branches whorl marks are visible at the positions from where branch or branches of higher order emerge during a particular flush. The portion of a branch between two successive flushes is called a shoot. The shoots from the current whorl of all the branches bear one leaf and a stipule at the base of the petiole. Of these, one is the extension of the parent branch and the rest form branches of higher order. During the next flush, these stipules break and shoots bearing a leaf and a stipule emerge. As before, one from each stipule is an extension of the parent branch and the remaining are branches of higher and further higher orders. This is followed during each flush. However, sometimes, for higher order branches, no branch appears with further higher order; only the parent branch continues to extend during successive flushes.

From the above, it is quite clear that a tree architecture undergoes certain changes during each flush. Thus a method of ordering which is able to consider these changes from flush to flush is preferred. Its ability to identify the position of the shoots at different canopy levels is an additional quality. As mentioned earlier, instead of branches, individual shoots are considered as the final unit of ordering. Shoots of different flushes can be distinguished easily with the whorl marks formed at the place of emergence. First, we classify the shoots with respect to the order of the branch to which they belong. However, this is not just sufficient as there is more than one branch of that particular order. To locate the exact position of a shoot we make use of the fact that the n th order branch emerges from a particular position of $(n - 1)$ th order, which again comes from an $(n - 2)$ th order and so on. Symbolically, we write $p_{ijk} \dots$, (figure 1a) to denote the order of a shoot, where i stands for the position of the shoot on the first order branch, j for the same on the second, k for the third and so on. The number of suffixes depends on the highest order of branches present in a tree. Some of the suffixes may be zero as well, viz p_{2100} (figure 1a). Obviously no non-zero suffix can follow a zero suffix.

In some cases, a shoot may produce more than one shoot of the next higher order. For example, p_{220} (figure 1b) produces three shoots. Clearly, one is p_{230} and the other two are p_{221} . To identify them distinctly we make an extension of the above notation by introducing a series of super suffixes— a, b, c, \dots and we write $p_{ijk}^{abc} \dots$ which denotes i th shoot of a th first order branch, j th shoot of b th second order branch and so on. And accordingly the shoots with order P_{221} can now be identified separately as p_{221}^{111} and p_{221}^{112} . This