SHORT COMMUNICATIONS

DETERMINATION OF UNI- AND TRIFOLIOLATE LEAF FORM IN FENUGREEK*

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The leaves of the Trifolieae are trifoliolate except for the first which usually is single (unifoliolate). Hereditary deviations from this pattern have recently been described by Bingham (1966).‡ He described *Medicago sativa* L. plants in which all leaves were unifoliolate, and others in which all leaves, including the first, were trifoliolate. This communication reports some observations on fenugreek, *Trigonella foenum-graecum* L., which bear on the physiological conditions favouring one or the other developmental pathway.

Embryo Culture

One series of observations stems from efforts to culture fenugreek embryos. In the mature seed the only primordium normally present is that of the first leaf, which is unifoliolate (Plate 1, Fig. 1). This primordium is initiated rather early during seed development. A protuberance was seen at the apex of an embryo of 1·0 mg dry weight but not on one of 0·5 mg, whereas the weight of a mature embryo was 12·5 mg. However, before primordium formation can be discerned, embryos are already bent but the cotyledons are not yet half way into the cotyledonary space of the ovule. Embryos of about this stage were explanted on an agar medium, with nutrient salts, 4% sucrose, and 2 mM L-arginine, and left for 2 weeks at room temperature. The embryos were somewhat elongated with cotyledons opened apart but not growing well, leaving viability in doubt. The first leaf primordium was present but it was trifoliolate in all the 25 embryos inspected. In eight of the embryos the second leaf primordium was far enough advanced to show that they would become trifoliolate (Plate 1, Fig. 3). In a later series of embryo cultures transitions were found: of 41 embryos, 28 had trifoliolate, 9 trilobed, and 4 simple first leaves. It is assumed that these embryos at explantation were at a critical stage, and that some were too far advanced to respond to explantation with trifoliolate development.

Some variations of these experiments seemed to indicate that, insofar as conditions allowed some *in vitro* growth, these would yield a trifoliolate first leaf. No marked difference in the incidence of trifoliolate development was apparent between embryos cultured in the continuous light of a fluorescent tube, or in continuous darkness. When cultures grown in light, with and without 2 mM L-arginine, were compared, there was a difference in colour and rate of development: those grown with L-arginine were light green, and further advanced, as indicated by the state of the

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‡ Bingham, E. T. (1966).—*Bot. Gaz.* **127**, 221.

primordia, than those grown without L-arginine which were yellow. Nevertheless, after a fortnight, of 20 embryos cultured in the absence of arginine, 13 were far enough advanced to allow the decision that 9 of them would become trifoliolate, 2 trilobed, and 2 single. However, substituting mannitol (2%) for the sucrose and at the same time omitting the arginine resulted in early death of all embryos. Similarly, a condition of anaerobiosis, although allowing the embryos to stay green, did not permit sufficient development for primordium formation.

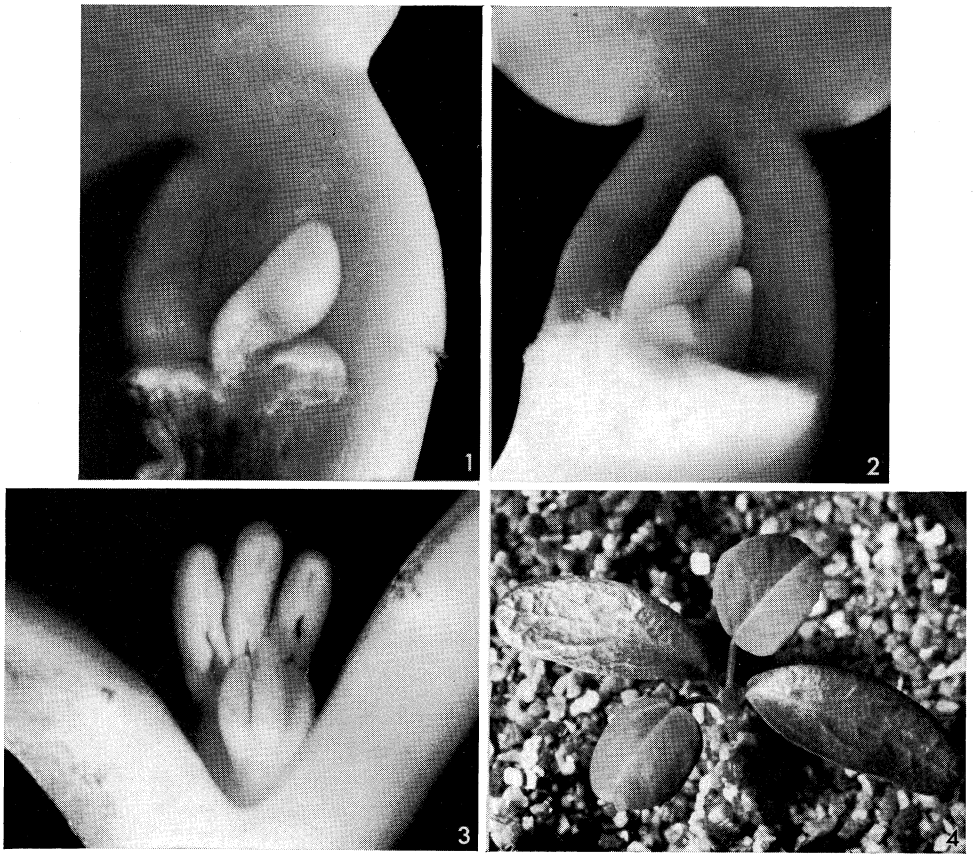
Intra-ovular Development

A different set of observations lends support to a correlation between intra-ovular development of the leaf primordium and the unifoliolate condition. As mentioned, fenugreek embryos usually have one leaf primordium at maturity. It was found by chance that the seeds of a particular plant contained a high percentage of embryos with two leaf primordia (Plate 1, Fig. 2). This seemed to be a hereditary trait because four plants grown from such seeds produced respectively 47, 48, 60, and 64% seeds with two leaf primordia in the embryo as compared with only two such embryos recorded in the 299 seeds from seven plants grown simultaneously from the common seed source. The interest here lies in the fact that the first two leaves of the seedlings from the seed with two leaf primordia were unifoliolate (Plate 1, Fig. 4), and that only primordia formed later, after germination, became trifoliolate.

Comment

The above observations taken together seem to suggest that a switch exists to the uni- or trifoliolate pathway of primordium development and that the setting of the switch corresponds to physiological conditions in the intra- and extra-ovular situation. On the one hand explantation of the young embryo conferred trifoliolate development in the first primordium; on the other hand, even the second primordium became unifoliolate when it formed inside the ovule. This suggestion does not attribute the trifoliolate development of the first leaf in the explanted embryo to a specific morphogenetic substance in the medium. The experiments suggest that light was not involved. Perhaps there is a difference in the state of aerobiosis between intra- and extra-ovular development, but exclusion of oxygen made the cultures fail. Explantation evokes general changes in the type of growth of the embryo, including increased expansion, opening of the cotyledons, elongation or swelling of hypocotyls, and sometimes root extension analogous to that in germination. However, in contrast to normal germination, the changes often resulted in distortion of form and discolorations. Germination and explantation may be envisaged as entailing a new metabolite or hormonal status which communicates with the apex and, in fenugreek, releases there the specific genetic expression of leaf form. This condition may perhaps govern leaf shape in other legumes in which one or more of the earlier leaves shows a similar contrast with later ones. However, it was noticed that in mature embryos of *Trifolium subterraneum* the second leaf was present as a trifoliolate primordium. On the other hand, although moribund, a young embryo of *Medicago orbicularis*, explanted on the arginine-containing medium, developed a trifoliolate first leaf primordium.

LEAF FORM IN FENUGREEK



Figs. 1 and 2.—Mature embryos of *Trigonella foenum-graecum* with one cotyledon removed showing one (Fig. 1) and two (Fig. 2) unifoliolate leaf primordia.

Fig. 3.—Embryo of *T. foenum-graecum*, cultured as described in text, showing, between diverging petioles, first and second leaves as trifoliolate.

Fig. 4.—*T. foenum-graecum* seedling germinated from seed with two leaf primordia, showing the first two leaves as unifoliolate.