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NITROGEN REGIME AND ISOENZYME CHANGES IN *VICIA FABA*

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Key Word Index—*Vicia faba*; Leguminosae; field bean; isoenzymes; esterase; glutamate–oxaloacetate transaminase; soluble proteins.

Abstract—Plants of *Vicia faba* were supplied either with fertilizer inorganic nitrogen or they received only nitrogen fixed by their root nodules. The esterase and transaminase isoenzyme profiles and the total water-soluble protein complement of seed cotyledons and of pollen did not vary irrespective of the N regime employed, whereas those of the leaf did. Possible causes and the implications of these differences are discussed.

INTRODUCTION

Previous papers [1, 2] have described the use of dry seed cotyledon and pollen non-specific esterase (EST: EC 3.1.1.2.) and glutamate–oxaloacetate transaminase (GOT: EC 2.6.1.1.) isoenzymes for the identification of *Vicia faba* L. inbred lines, and for measuring crossing between them. Both these papers reported isoenzyme patterns which are invariant under a range of environmental conditions, and furthermore because both dry seeds and pollen are dormant stages in the life history of the plant, their genotype-specific patterns are not confused by ontogenetic changes.

The present paper describes the effect of contrasting nitrogen regimes on the EST, GOT and water-soluble protein complements of seed cotyledons, pollen and leaves of *V. faba*.

RESULTS

The patterns of EST, GOT and soluble protein extracted from seed cotyledons and pollen showed no change over all the nitrogen treatments employed, other than slight variations in band intensities (Fig. 1).

In contrast, patterns of leaf proteins and EST isoenzymes were clearly dependent on the nitrogen regime to which the plant was exposed. When compared to the nodulated plants, which received no additional inorganic nitrogen, all other treatments (see Experimental) produced pronounced differences in EST and protein band intensities to this control. Most pronounced was the different pattern from non-nodulated plants which did not receive inorganic N where three additional protein and three additional EST bands were found (Fig. 2).

Protein and EST patterns of leaves from the first flowering node, of nodulated plants without added inorganic nitrogen extracted at flower initiation, flower anthesis, and early pod set revealed that additional bands appear throughout leaf development (Fig. 3). The patterns in leaves sampled at early pod set resembled those seen from leaves of non-nodulated plants receiving no added inorganic nitrogen sampled at flower initiation.

DISCUSSION

The stability of the seed and pollen isoenzyme patterns under contrasting nitrogen regimes confirms the value of sampling physiologically dormant tissues if comparisons between genotypes are to be made. Pea cotyledon albumins and globulins have been shown to be stable under varying nitrogen treatments [3], and *Phaseolus vulgaris* seed proteins have also been demonstrated to be unaffected by changes in nitrogen fertilization, climatic conditions and soil properties [4]. Similarly, seed EST patterns of wheat [5] and peanuts [6] have been shown to be constant in different environments and harvest years.

In contrast, numerous studies have revealed that, in vegetative tissues, isoenzymes are not only subject to pronounced developmental changes, but are also sensitive to the environmental fluctuations prevailing during

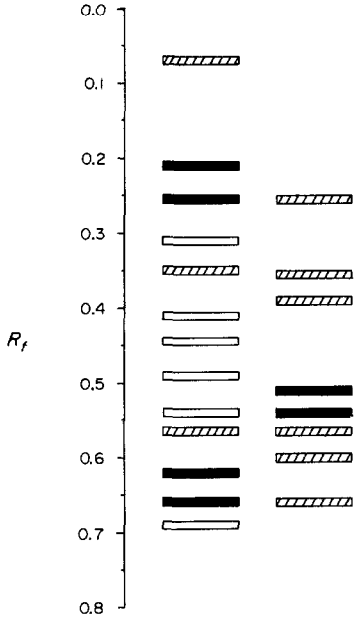

Fig. 1. Seed (left) and pollen (right) EST patterns of *V. faba* inbred line 51/3, separated on 7% acrylamide gel. Zymograms showed only slight variations in band intensity over all experimental treatments. The density of shading is proportional to the band staining intensity in all 3 figures.

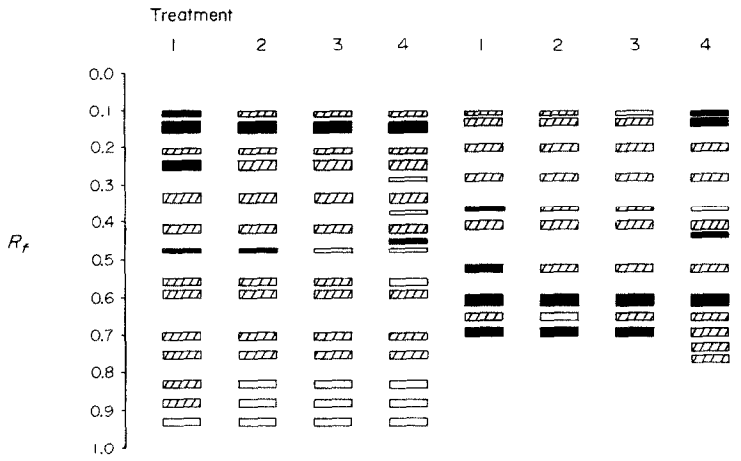

Fig. 2. Total soluble protein (left) and EST isoenzyme patterns (right) of leaves of *V. faba* inbred line YT 11/2. For details of treatments see Experimental.

plant growth. A period of only four days of nitrogen stress was sufficient to induce changes in isoenzyme activity and composition in cucumber leaves [7]. Again, for cultivar identification in *Agrostis palustris* and *Poa pratensis*, it was essential that plant material be grown under uniform temperature, light, moisture and nutritional conditions prior to leaf sampling [8]; these requirements are often difficult to fulfil and render large scale sampling uneconomic.

The additional EST and protein bands observed in leaves of non-nodulated plants grown with low levels of inorganic N could have arisen either by the induced synthesis of additional proteins, or by the deficiency of nitrogen shifting the developmental cycle of the leaf more rapidly towards senescence. The results presented in Fig. 3, showing an increase in number of EST bands through leaf development, support the latter interpretation, and are also in accordance with reports that additional EST bands occur in senescing leaves of *Festuca pratensis* [9]. Lack of nitrogen in *V. faba* may have resulted in early activation of leaf enzymes for the mobilization of metabolites in leaves sampled at the end of the vegetative period of growth, to meet the requirements for reproductive growth, so that in the absence of adequate root-derived nitrogen, nitrogenous compounds were rapidly recycled during plant development. It has been shown [10] that soyabean lines which exhibit prolonged nitrogen fixation also show a delay in leaf sensescence, thus linking this with nitrogen status, and in this species it is known that different rates of leaf sensescence occur depending on whether plants receive nitrogen from fixation in root nodules, or as nitrate [11].

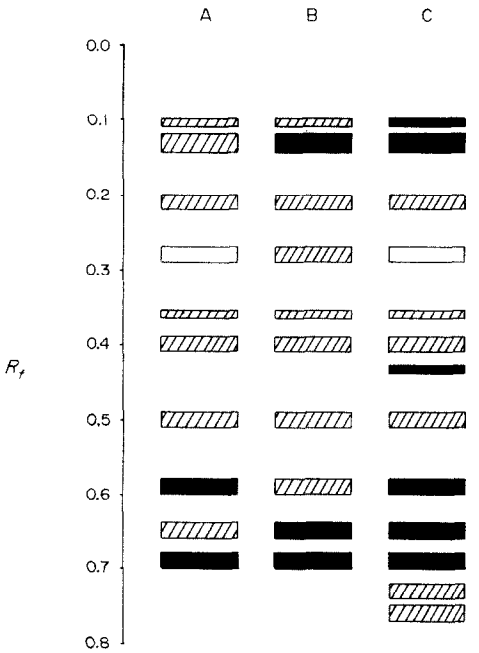

Fig. 3. Leaf esterase zymograms from the first flowering node of nodulated *V. faba* plants (inbred line YT 11/2) receiving no additional nitrogen and sampled at (A) early flower bud formation, (B) anthesis and (C) early pod formation.

In 1967, Hart and Bhatia [12] concluded that leaf tissue was unsuitable material for interspecific comparisons of protein profiles of *Nicotiana*, due to its sensitivity to environmental conditions, and found that environmentally-induced changes exceeded interspecific differences. The results presented here for *V. faba* confirm these reservations.

EXPERIMENTAL

Materials. Inbred breeding lines of *V. faba* were obtained as previously described [1].

Growth conditions. Seeds of inbred lines 224 and 51/3 were surface sterilized in 2% NaClO soln, and sown in sterilized Levington compost in 5″ plastic pots. Half of the pots were inoculated with soil in which well-nodulated *V. faba* plants had previously been grown, and half of these in addition received 200 ml of a complete nutrient feed containing 154 ppm nitrogen (NO_3) twice weekly. Non-nodulated plants were either given inorganic nitrogen, as described above, or grown with no nitrogen other than that present in the cotyledons and sterile compost. The four treatments were thus: (1) +nodules, no inorganic N (control); (2) +nodules, +inorganic N; (3) no nodules, +inorganic N; and (4) no nodules, no inorganic N. All plants were regularly watered with distilled water, and were grown under greenhouse conditions as described in ref. [1].

Extraction. Seeds and pollen were extracted as described in ref. [1]. Leaves, harvested from the first flowering node at the period of transition between vegetative and reproductive growth, were extracted by the method of Gerbrandy and Verleur [13]. Preparation of gels was as described in [1].

Enzyme assay and protein staining. EST and GOT were assayed as described in ref. [1]. Proteins were stained by immersing gels in a 0.025% (w/v) soln of Coomassie Blue R250 in 50% MeOH and 7% HOAc overnight, and then destaining in successive changes of 25% MeOH containing 7% HOAc.

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