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Genetic and physiological influences on differentiation in tissue cultures of a legume, *Lathyrus sativus*

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Summary. Callus initiation and subsequent organogenetic potential in cultures of shoot meristems of *Lathyrus sativus* is under genetic control. This genetic influence is observable not only at the broader intercultivar level, but also between genotypes descended from the same cultivar. However, it is possible to achieve higher levels of morphogenetic response even in recalcitrant genotypes by using the physiologically altered explant. On activation of the dormant lateral bud meristems consequent to decapitation of the apical bud, it is possible to induce organogenesis in the tissues of all genotypes. Callus masses arising from the shoot meristems and leaf explants of cv. 'LSD-3' also exhibit somatic embryogenesis which results in the development of mature plantlets under culture conditions.

Key words: Genotype – *Lathyrus sativus* – Plantlets – Somatic embryogenesis – Tissue cultures

Introduction

Successful application of tissue culture technology to the improvement of crop plants depends upon the ability to regenerate plants in vitro. Though the number of species amenable to such experimentation is constantly increasing (Murashige 1978; Vasil and Vasil 1980; Krikorian 1982), many plants such as legumes and other important crops have remained relatively refractory.

One of the major problems encountered during such investigations with crop plants is the selection of proper genotypes possessing high morphogenetic potential. Unfortunately, too little attention has been paid in the past in overcoming the genetic barriers by physiological means, and instead conventional, long-term, tedious breeding procedures have been employed in such plants as alfalfa (Bingham et al. 1975), potato (Jacobsen and Sopory 1978), and red clover (Keyes et al. 1980). The present work was initiated in order to investigate the extent of genotypic and physiological influence on the morphogenetic potential in *Lathyrus sativus,* an important seed legume of India.

Materials and methods

Eight genotypes of *Lathyrus sativus,* namely 'Sankoli', 'Rewa-2', 'RL-31', 'RPL-29' 'P-263', 'P-24', 'LSD-1' and 'LSD-3', obtained from Raipur Agriculture College, Madhya Pradesh, India, were employed in the present investigation. The growth conditions for all the genotypes were similar to those described earlier (Gharyal and Maheshwari 1980). The leaf explants and shoot meristems were taken from plants 10–15 days old. However, during the present investigation, experiments were conducted with three types of shoot-bud meristems, namely (i) apical meristem, (ii) axillary-bud meristem, and (iii) the activated-axillary-bud meristem (i.e. the 3–4 day old activated-axillary meristem which results as a consequence of decapitating the apical bud). A minimum of 48 cultures were raised for each treatment.

The culture conditions were also similar to those described earlier (Gharyal and Maheshwari 1980). In the case of leaflet explants, the B_5 medium containing NAA (2 mg/l) + BAP (0.5 mg/l), though satisfactory for initiation of callus, was not suitable for long-term maintenance of cultures, and for routine subculture a medium supplemented with IAA (0.5 mg/l) + BAP (1 mg/l) was employed. Therefore, the shoot bud meristems were directly inoculated onto this medium.

Results

Shoot meristems

The various shoot and bud meristems showed callusing within 2 weeks of culture in all of the eight genotypes

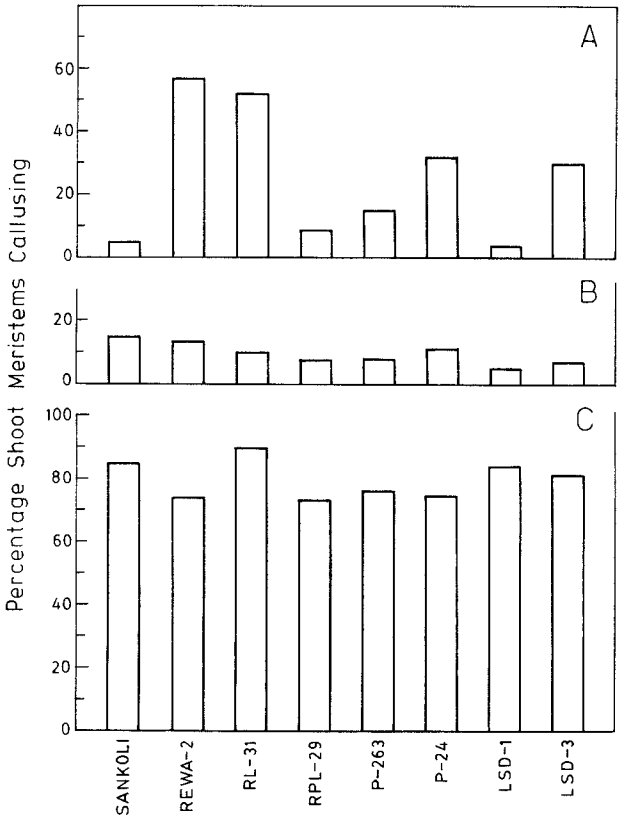

Fig. 1 A–C. Comparative response in callusing of shoot meristems of eight genotypes of *Lathyrus sativus;* **A** apical meristem, **B** axillary-bud meristem, and **C** activated-axillary-bud meristem. Observations were taken after a month of culture

tested. Quantitatively, considerable variation was observed in the response of the apical meristems, indicating a strong genotypic influence (Fig. 1 A). The axillary-bud meristems showed an overall low response (Fig. 1 B) whereas the activated-axillary-bud meristems showed such high percentages of callusing (Fig. 1 C) that the influence of genotype was not apparent. Furthermore, the number of cultures differentiating shoot buds was also much higher in the activated-axillary-bud meristems than in the apical-bud and dormant bud meristems (Table 1).

Although the shoot meristem cultures were not maintained for long periods in all the genotypes, those of cv. 'LSD-3' – in which differentiation via organogenesis was reported (Gharyal and Maheshwari 1980) – were routinely subcultured on B_5 medium supplemented with IAA (0.5 mg/l) and BAP (1 mg/l) for more than two years. After 4 or 5 subcultures (each of 6 to 8 week duration), shoot formation was inhibited. Interestingly, however, the cultures contained numerous embryoidal structures. Small plantlets could be obtained from these cultures on BM + NAA (2 mg/l) + BAP (0.5 mg/l). Further attempts at obtaining mature plantlets met with varying degrees of success, i.e. sometimes only roots developed further, sometimes only shoots (Fig. 2 D, E) and at other times the plantlets callused. Nevertheless, on half-strength B_5 medium supplemented with 1 mg/l NAA, normal mature plantlets could be obtained (Fig. 2 F).

Leaflet explants

In view of the importance and utility of differentiation from mature leaf tissue, attempts were also made to initiate, maintain, and differentiate leaf cultures. Leaflets from the eight genotypes were cultured on B_5 + NAA (2 mg/l) + BAP (0.5 mg/l). Unlike for shoot calli, no genotypic influence was observed in these cultures. In fact, a high percentage of cultures (70–90) showed callus initiation and 'nodulation' (Fig. 2 A) within 2 weeks in all the eight genotypes. Frequent differentiation of roots was also observed on this medium.

Callus arising from leaflets of cultivar 'LSD-3' was maintained on medium containing 0.5 mg/l IAA and 1 mg/l BAP. During the initial 3–4 subcultures, these cultures showed frequent formation of shoots (Fig. 2 B), which sometimes even bore flower buds, but never developed a root. However, on subsequent subculture, differentiation of embryoids was observed (Fig. 2 C). Normal mature plantlets could be obtained when the young embryoidal plantlets with a defined root-shoot axes were subcultured on a medium supplemented with IAA (2 mg/l) + coconut milk (15%; v/v) (Fig. 2 G).

Discussion

Lathyrus sativus, a highly nutritious pulse legume of the semi-arid regions, is widely cultivated in India. Unfortunately, due to the presence of β-N-oxalyl-L-α,β-diaminopropionic acid, its consumption leads to neuro-

Table 1. Comparative data on the morphogenetic response of the apical meristems and the activated-axillary-bud meristems, scored after two months of culture. The morphogenetic response of the axillary meristems was negligible

Genotype	Percentage morphogenetic cultures	
	Apical meristem	Activated-axillary-bud meristem
SANKOLI	17	83
REWA-2	67	67
RL-31	67	92
RPL-29	17	67
P-263	42	67
P-24	75	97
LSD-1	17	75
LSD-3	17	75

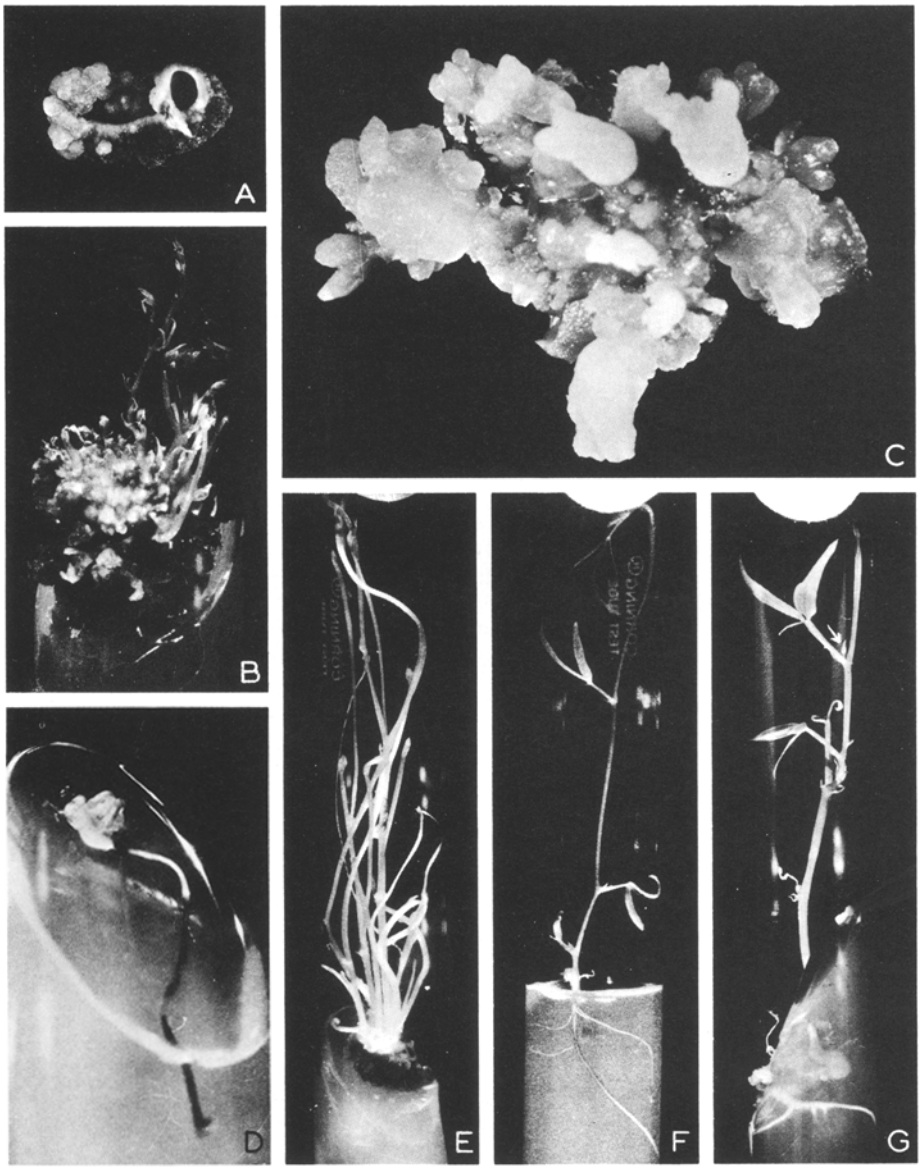

Fig. 2 A–G. Differentiation in tissue cultures of *Lathyrus sativus* cv. 'LSD-3'. **A** a 2-week-old leaflet explant showing callusing and nodulation, **B** a third passage leaflet culture showing well developed shoots, **C** same with embryoids, after fifth passage, **D–F** growth of stunted plantlets from shoot meristem cultures, with an elongated root on BM (**D**), or shoots alone on BM+0.5 mg/l IAA (**E**), and normal plantlet on ½ BM+1 mg/l NAA (**F**), **G** well differentiated plantlet from leaflet cultures with flower bud (*arrow*) on BM+2 mg/l IAA+15% CM (v/v)

lathyrism. Thus, attempts are being made to develop cultivars with decreased levels of this toxic amino acid (Zenk 1974). In an effort directed towards their further improvement utilizing the in vitro manipulation technique, one wild type and seven improved genotypes were tested for their tissue culturability and a strong genotypic influence was observed (Fig. 1). Nevertheless, the present work shows that it is possible to overcome the inherent inhibitory effect of the genotype by altering the physiological status of the donor plant. We have thus developed a new approach for development of newer varieties of this pulse for economic purposes. How decapitation of apical meristems induces higher response in axillary buds is still not clear. However, it is well known that such treatment leads to increase in auxin concentration. Though the role of other factors cannot be ruled out, higher auxin content can bring about further changes in metabolism leading to higher morphogenic response.

In any case, similar genotypic differences – in in vitro manipulability of tissue – as reported here, have been observed earlier in *Medicago sativa* where it is present at not only the generic, species and varietal levels, but even within different plants of the same cultivar (Kao and Michayluk 1980). Finally, as shown in the present investigation, it is also possible to induce somatic embryogenesis in leaf and shoot meristem cultures of the cultivar 'LSD-3'. Differentiation via

somatic embryogenesis in legumes from leaf explants has also been recently observed in *Trifolium pratense* and *Medicago sativa* (Phillips and Collins 1980; Kao and Michayluk 1981), and where again it is reported to be under genetic control.

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