IN VITRO DEVELOPMENT OF BUDS, FLOWERS AND PODS OF OILSEED RAPE

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INTRODUCTION

Losses through pod and seed abortion can be very important in oilseed rape and are usually attributed to insufficient assimilate supply (Mendham, Russell and Buzza, 1984; Leterme, 1983). However, an alternative explanation is an abortion induced by phytohormones, possibly ABA or auxin, resulting in a loss of sink strength (Pechan and Morgan, 1985). In the present study, *in vitro* cultures were used in order: (1) to test whether floral organs can develop successfully *in vitro* depending on their developmental stages; (2) to assess the influence of phytohormones, particularly abortion-inducing hormones (ABA and auxin) on pod and seed set and development under various nutritional conditions and (3) to examin the influence of nutrients on pod and seed set and development.

MATERIALS AND METHODS

Plant Material

Spring rape plants (cv Haplona) were grown in individual pots under greenhouse conditions.

Culture Method

The basal medium (BM) was prepared with Murashige and Skoog's minerals and vitamins at half concentration, sucrose 30g/l and distilled water, pH adjusted to 5.8. Universal bottles (25ml) were filled up, labelled, sealed with aluminium foil and sterilised in a pressure cooker. Plant growth substances (PGS) were added from concentrated stock solutions. The explants consisted of a segment of stem with 4 opening flowers taken from the lower part of the terminal raceme. After sterilisation, the stem was inserted in a bottle and the explant were cultured 8-10 days in an incubator (20 C, continuous light). At harvest, the length, fresh weight and dry weight of the pods were measured and their number of seeds was determined using X-rays (Ancha, 1989).

Experiments

Preliminary experiment Explants were cultured on the basal medium from the following stages: bud 3mm in length (B3), bud 5mm in length (B5), flower opening (F) and 3 day-old pod (P); 3 explants were harvested after 5, 10 and 15 days of culture as described above.

Influence of phytohormones under high nutrient supply Benzyl Amino Purine (BAP), Naphtyl Acetic Acid (NAA) and Gibberellic Acid (GA) were added singly to the basal medium to

give final concentrations of 10-7, 10-6 and 10-5 M. A similar experiment was made separately with Abscissic Acid (ABA).

Influence of NAA and ABA under low nutrient supply ABA and NAA at 10-7, 10-6 and 10-5 M were added singly to a basal medium containing 1 g/l sucrose.

Influence of nutrients supply The following combinations were used. Minerals and vitamins: 0 and Murashige and Skoog's half concentration. Sucrose: 1, 10, 30 and 50 g/l.

RESULTS

Preliminary Experiment (Fig.1)

The B3 explants did not grow; about half of the B5 explants developed flowers and small pods containing few seeds. The F and P explants developed pods which looked normal and contained about 9-10 seeds. Pod elongation was rapid during the first days (after a lag-period for the F-explants) and stopped when the pods were 12-13 days old. Pod weight increased steadily at first and more rapidly when pod elongation stopped. Pod length and weight were strongly correlated to seed number and the regression between seed number and pod length was not different from that reported *in vivo* (data not shown).

Influence of phytohormones under high nutrient supply (Table 1)

The number of seeds per pod was not affected by NAA, GA or BAP. Pod elongation was however enhanced by high levels of GA. Pod dry weight was increased by low BAP concentration (10-7M), unaffected at 10-6 M and depressed at 10-5 M, and also by high levels of GA (10-6 and 10-5 M).

ABA had no significant effect on seed set, pod length or weight, though seed abortion tended to increase at high ABA levels.

In both experiments, no pod abortion was observed.

Influence of ABA and NAA under low nutrient supply (Fig.2)

ABA and NAA had no significant effect on seed abortion, though the pods grown on high ABA tended to have fewer seeds. No pod abortion was observed (data not shown). Pod length and weight decreased gradually with increasing ABA concentration, and the pods grown on ABA 10-5 M were significantly shorter and lighter than the control. Pod length and weight were also depressed by NAA, but without dose-response effect.

Influence of nutrient supply (Fig.3)

Pod abortion remained extremely low for all the treatments (data not shown), but pod growth and seed set depended strongly on the level of both minerals and sucrose.

In absence of minerals: on low sucrose, the pods started to develop but remained small and many seeds were degenerating

after a few days. When more sucrose was supplied, the pods accumulated more dry matter but pod elongation and seed growth were inhibited.

In presence of minerals: on low sucrose, the development of the pods was similar to that without minerals. At 1 % sucrose, pod length, weight and seed numbers were significantly enhanced and the pods were very similar to attached pods. At 3% sucrose, pod weight increased further but pod length and seed number did not; at 5% sucrose, pod and seed growth were inhibited and showed symptoms of water stress.

DISCUSSION

The inability of the buds to develop on a basal medium suggests strongly that they depend on maternal hormones - possibly gibberellins and/or auxin (Bouttier and Merrien, 1991) - for their organogenesis. This dependence could provide important control points for the mother plant, particularly when ovule fertility is being determined. In contrast, the flowers and pods were able to develop with a seemingly normal pattern, i.e. elongation during the first days followed by the onset of rapid dry matter accumulation, and the same quantitative relationship between pod length and seed number as *in vivo*.

Phytohormones added to the medium elicited only small quantitative effects on pod growth and had no significant influence on seed set. These results are in agreement with those obtained *in vivo* (Ancha, 1989; Bouttier, unpublished) and have the following implications: (1) the phytohormones necessary to pod and seed development originate from within the pods; (2) the hormonal independence of the flowers and pods contribute probably to a high sink strength compared to the buds, and (3) though high levels of ABA under low nutrient supply may increase seed abortion, hormonal factors are unlikely to be important in the determination of seed set. Furthermore, the marked influence of nutrient levels on seed retention provides strong evidence that seed abortion *in vivo* is regulated by the supply of C-assimilates and minerals rather than by hormonal factors.

The absence of effect of both nutritional and hormonal conditions on pod set indicates that the abortion/retention of a pod is determined during bud development.

LITERATURE CITED

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Table 1: Influence of phytohormones under high nutrient supply on the pod growth and development in vitro

O, -, +: no significant effect (5%level), depressive and promotive effect compared to the basal medium, respectively. Concentrations in mol/l

	BAP			GA			NAA	ABA		
	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	all conc.	10^{-7}	10^{-6}	10^{-5}
Pod abortion	0	0	0	0	0	0	0	0	0	0
Seed no/pod	0	0	0	0	0	0	0	0	0	0-
Pod length	0	0	0	0	0+	+	0	0	0	0-
Pod dry wt	+	0	-	0	-	-	0	0	0	0

Fig. 1: Growth and development of buds, flowers and pods in vitro

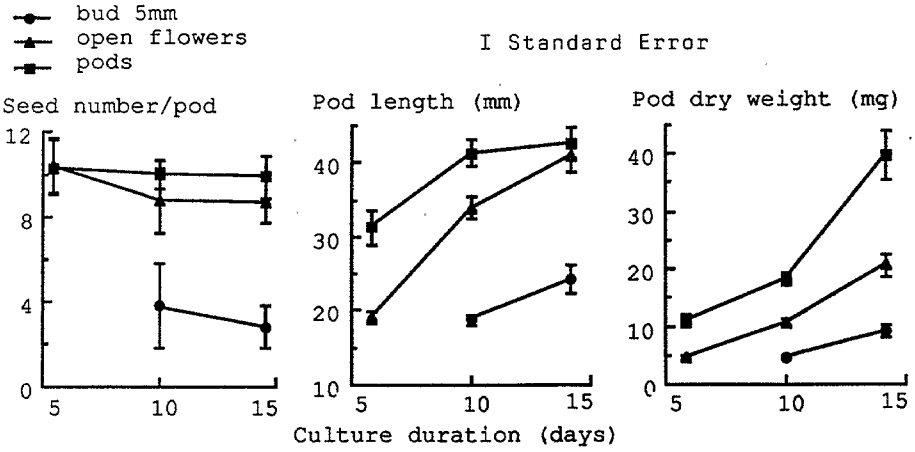

Fig 2: Influence of ABA and NAA under low nutrient supply on pod and seed development in vitro

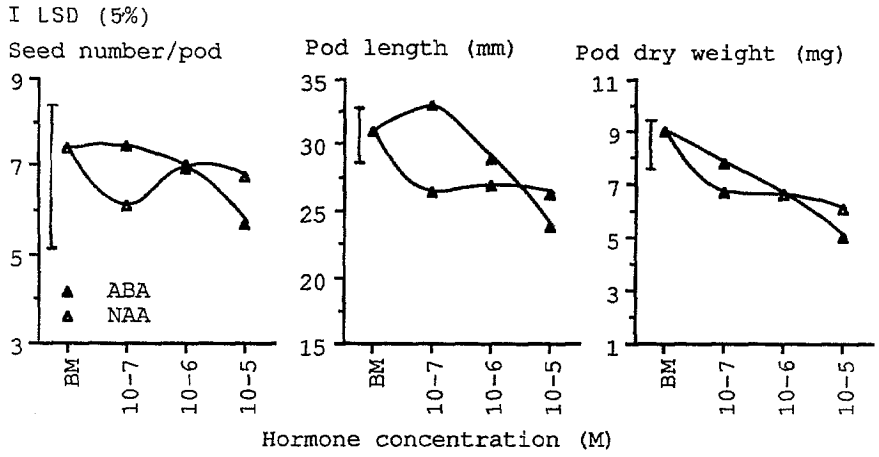

Fig 3: Influence of nutrients on pod and seed development in vitro