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Induction of Fruit Set and Development in Pea Ovary Explants by Gibberellic Acid

José L. García-Martínez and Juan Carbonell

Instituto de Agroquímica y Tecnología de Alimentos, C.S.I.C., Jaime Roig 11, 46010-Valencia, Spain

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Abstract. The response of unpollinated ovary explants of *Pisum sativum* L. cv. Alaska No. 7 to several plant growth regulators and nutrients has been studied. Explants consisted of a segment of stem and an emasculated flower with or without the adjacent leaf. They were made on the day equivalent to anthesis and were cultured in a liquid medium. Growth regulators were applied either in the solution or directly to the ovaries. Gibberellic acid (GA_3) in the presence of sucrose, but not indole-3-acetic acid or N^6-(Δ^2-isopentenyl)-adenine (2iP), induced fruit set and development of parthenocarpic fruits, the final length of these being a function of the intensity of the GA_3 treatment. The capacity of ovaries to respond fully to GA_3 was not lost after incubation of explants in water or 50 mM sucrose for 1 day and was similar in explants made between the day of anthesis and 3 days later. Limited growth was obtained with 100 mM sucrose alone but this effect was counteracted by 2′-isopropyl-4′-(trimethyl ammonium chloride)-5′-methylphenyl piperidine-1-carboxylate (AMO-1618). This inhibitor was ineffective when GA_3 was applied to the ovary. The development of the fruit was proportional to the length of the segment of stem up to 5 cm. The presence of the leaf in the explant enhanced the development of the fruit. These results indicate that a gibberellin is necessary for setting and development of fruits from cultured ovaries and that this effect depends on an appropriate source of nutrients. The course of development of parthenocarpic fruits on explants was similar to that of seeded fruits on the intact plant. The cultured pea ovary system offers convenient means to investigate the role of gibberellins and nutrients in fruit set and development.

Nitsch (1951) described techniques of fruit culture *in vitro* using explants retaining a small portion of pedicel. Ovaries from pollinated flowers of different

species developed under sterile conditions on a simple nutrient medium composed primarily of agar, sucrose, and mineral salts. In the case of tomato, limited parthenocarpic growth was achieved by adding synthetic auxins to the medium. The procedures of Nitsch, particularly the agar media, have been used to investigate the role of nutrients (Leopold and Scott 1952, Jong et al. 1974) and hormones (Jong and Bruinsma 1974, Peterson 1974, Kano and Asahira 1978) in the development of the ovary. The production of gibberellin (Baldev et al. 1965) and cytokinin (Van Staden and Button 1978) in seeds has been studied in cultured excised pea pods.

Pea plants have proved a useful material for studying the role of hormones in fruit set and development (García-Martínez and Carbonell 1980, Sponsel 1982). We have now developed a simple system to grow excised unpollinated pea ovaries retaining the entire pedicel and a segment of stem, in liquid medium under nonsterile conditions. We found that gibberellic acid (GA_3) induced the ovaries to grow rapidly into parthenocarpic fruits and that the development of these was quite similar to that of the seeded fruits on intact plants.

Materials and Methods

Seeds (multiplied from seeds purchased originally from Asgrow, Complejo Agrícola Semillas, Madrid) of pea (*Pisum sativum* L. cv. Alaska No. 7) were sterilized for 20 min in 2% NaClO, washed with deionized water, germinated and grown for 7 days in perlite under daylight conditions, and irrigated with deionized water. Seedlings were selected for uniformity and transferred into 11.5-cm diameter pots (1 plant per pot) containing perlite and automatically irrigated with Hoagland's No. 1 solution every hour. Other culture conditions were as described previously (Carbonell and García-Martínez 1980).

Only the first flower of each plant was used for the experiments. Flowers were emasculated 2 days before anthesis (day −2), and at anthesis (day 0) explants consisting of the ovary (about 9 mm long and about 15 mg weight), sepals, pedicel, a segment of stem (3 cm above and 7 cm below the node), and sometimes the leaf on the node, were made and placed upright in vials with solution. Every other day the explants were transferred in new vials with fresh solution. Nutrients were applied in the solutions and plant growth regulators either in the solution or directly to the ovary (20 µl of aqueous solution containing 0.1% Tween 80; as control ovaries treated with 20 µl of 0.1% Tween were used). The compounds used were: indole-3-acetic acid (IAA) (E. Merck, Darmstadt, Germany), GA_3 (Fluka AG, Buchs, Switzerland), N^6-(Δ^2-isopentenyl)-adenine (2iP) (Sigma, St. Louis, Missouri, USA), and 2′-isopropyl-4′-(trimethyl ammonium chloride)-5′-methylphenyl piperidine-1-carboxylate (AMO-1618) (Calbiochem-Behring Corp., La Jolla, California, USA); sucrose (extra pure), glucose, and fructose were from Merck. Explants were maintained on a photoperiod of 16 h light (fluorescent tubes Philips TLD 18w/33, about 9,000 lux at the level of the explants) (22–24°C) and 8 h dark (16–18°C). No apparent microbial contamination was observed in the solution even after 3 days in the culture medium. Final length and weight of the pods were measured after at least 6 days of incubation.

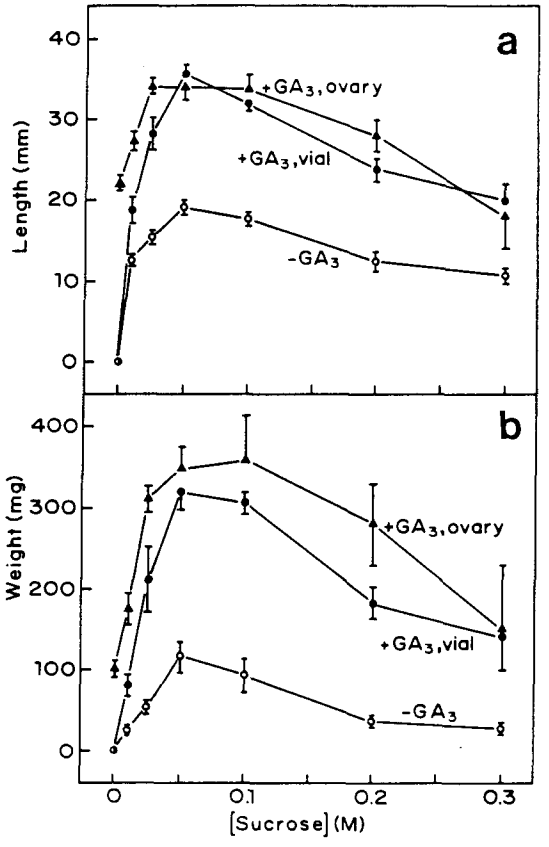

Fig. 1. Effect of GA_3 and sucrose on the length (a) and weight (b) of parthenocarpic pea fruits in explants without leaves. Each point represents the mean of five replicates ± SEM. Data were recorded on day 8. Fruit set was 100% except with no sucrose ($-GA_3$ and $+GA_3$, vial) (0 ovaries set), and 10 mM sucrose ($-GA_3$) (4 ovaries set). $+GA_3$, vial = 10^{-6} M GA_3 in the vial; $+GA_3$, ovary = 20 µl of 100 µg $GA_3 ml^{-1}$ per ovary.

Results

The application of GA_3 directly to the ovaries induced fruit set and development of unpollinated ovaries in explants without leaves cultured in water (Fig. 1). The development of GA_3-treated ovaries was dependent on the concentration of sucrose in the culture solution, attaining a maximum in the range of 25–100 mM. A similar effect was observed when GA_3 was applied in the solution, although in this case no fruit was obtained in the absence of sucrose. A limited growth of the ovaries was also induced by sucrose alone with a maximum at 50 mM. Similar results were obtained when growth was expressed either as maximal length (Fig. 1a) or as fresh weight (Fig. 1b) of the pod, although in the last case the differences between $-GA_3$ and $+GA_3$ treatments were greater (the ratio of lengths of $+GA_3$ and $-GA_3$ fruits cultured in 50 mM sucrose was 1.8, whereas the ratio of weights was 2.8). Sucrose could be substituted by glucose or fructose. Similar results were obtained with either 0.1 M sucrose or 0.2 M glucose or fructose. Other hormones like IAA and 2iP had no effect on fruit development when applied either in the culture solution (10^{-9}–10^{-4} M) or to the ovary (3×10^{-6}–3×10^{-4} M) in the presence of 100 mM sucrose.

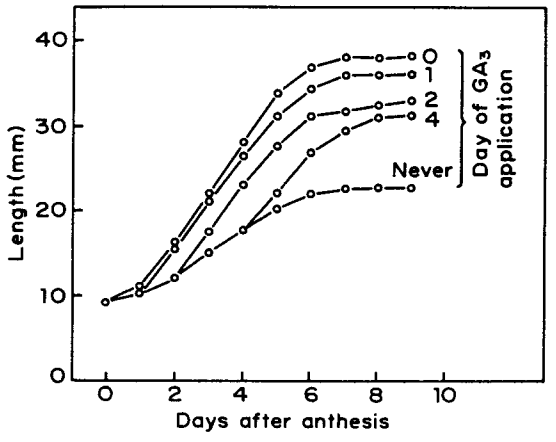

Fig. 2. Duration of the capacity of the pea ovary explants to respond to GA_3. Explants without leaves were made on day 0, incubated in 50 mM sucrose, and 20 µl of 100 µg GA_3ml^{-1} applied to the ovary after different times. Each point represents the mean of three replicates.

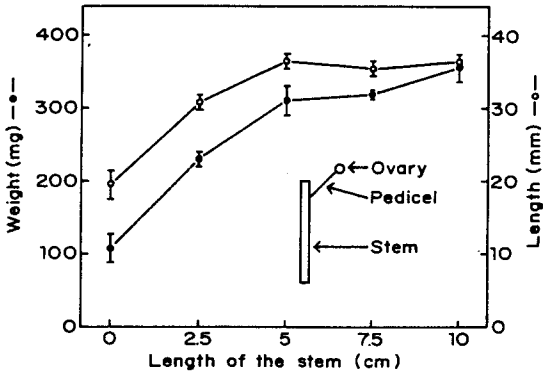

Fig. 3. Effect of the length of stem segment of explants without leaves on parthenocarpic pea fruit development. Fruit set was 100% in all the cases. Data were recorded on day 7. Each point represents the mean of four replicates ± SEM. The vials contained 50 mM sucrose and the ovaries were treated with 20 µl of 100 µg GA_3ml^{-1}.

The response of the ovary to GA_3 and sucrose was practically the same when explants were made between day 0 and day 3 and GA_3 was applied in the solution at the excision. Significantly shorter fruits were obtained with explants made on day −2 (data not shown).

In order to determine the duration of the capacity of the explanted ovaries to respond to GA_3, they were placed in 50 mM sucrose immediately after excision and treated with GA_3 at different times. Figure 2 shows that 24 h preincubation in sucrose did not affect significantly the subsequent course of development and the final length of the pod. Later applications of GA_3 resulted in retarded development and progressively smaller ovaries.

The stem had a dramatic influence on the growth of the explanted ovary, as shown in Fig. 3. The final size of the pod was proportional to the length of the stem segment between 0 and 5 cm (between 0 and 7.5 cm with 10^{-6} M GA_3 applied in the solution), and practically no additional response was obtained with longer segments. No significant differences in the development of the pod were obtained between explants made with 10 cm of stem retaining 10, 7, or 5 cm below the node. The effect of the stem could not be substituted by nutrients like KCl, $CaCl_2$, KNO_3, KH_2PO_4, or amino acids (glycine, glutamine, asparagine, and arginine) at 10 mM (data not presented).

Table 1. Influence of the leaves on the development of pea ovary explants in the presence or absence of sucrose.

		$-GA_3$		$+GA_3$[a]	
		− sucrose	+ sucrose[b]	− sucrose	+ sucrose[b]
Length (mm)	− leaves	—	15.9 ± 1.3	21.5 ± 2.0	28.8 ± 0.8
	+ leaves	24.9 ± 1.4	25.8 ± 0.4	38.5 ± 2.5	41.0 ± 1.0
Weight (mg)	− leaves	—	86 ± 18	66 ± 6	201 ± 12
	+ leaves	140 ± 11	192 ± 19	264 ± 23	527 ± 41

Data are the means ± SEM of two independent experiments, four replicates per experiment. Results were recorded on day 8.
[a] 20 µl of 100 µg $GA_3 ml^{-1}$ per ovary.
[b] 100 mM sucrose in the vials.

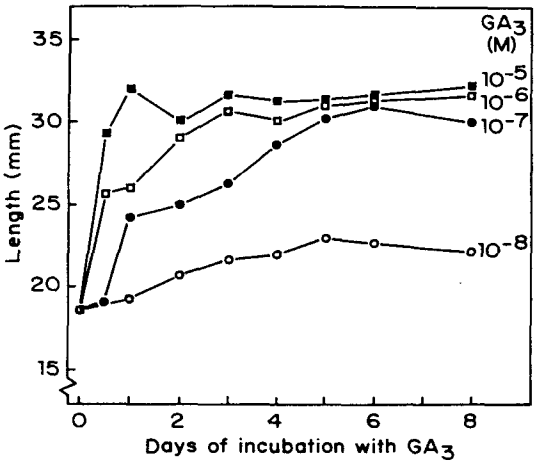

Fig. 4. Effect of the length of GA_3 treatment on development of pea fruits on explants without leaves. Explants were made on day 0 and incubated in 100 mM sucrose plus different concentrations of GA_3 in the vials and transferred to 100 mM sucrose after different times. Each point represents the mean of three replicates.

The leaf adjacent to the ovary enhanced markedly the development of fruits from ovaries treated or not with GA_3 in explants cultured in the presence or absence of sucrose (Table 1). Fruits on explants with leaves in the absence of sucrose were bigger than fruits on explants without leaves in the presence of sucrose. Whereas no significant differences could be observed between the length of fruits on explants with leaves in the presence or absence of sucrose, the weight of the former was significantly greater.

When GA_3 was placed in the culture medium, the effect of GA_3 on the development of the ovary was a function of the length of GA_3 treatment, and the lower the concentration of GA_3 the longer the time of incubation in GA_3 necessary to obtain the maximum effect (Fig. 4). For instance, with 10^{-5} M GA_3, saturation was attained with a treatment of 12–24 h, whereas with 10^{-8} M GA_3, saturation was attained only after 5 days of feeding. The final size of the fruits was proportional to GA_3 concentration between 10^{-8} and 10^{-5} M. This effect was particularly clear within short periods of incubation in GA_3 solution (up to 4 days). When GA_3 was applied directly to the ovary in explants with or without leaves, the length of fruits was proportional to the GA_3 con-

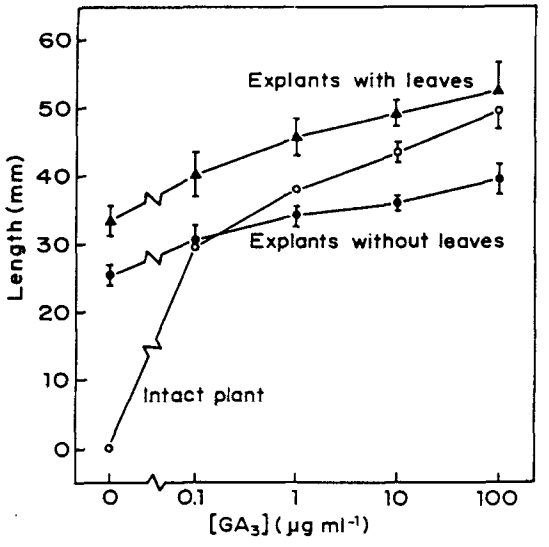

Fig. 5. Effect of GA_3 concentration on the development of parthenocarpic pea fruits on the intact plant and on cultured explants with and without leaves. The ovaries were treated with 20 μl of 100 μg GA_3ml^{-1} and explants cultured in 100 mM sucrose. Each point represents the mean of four replicates ± SEM. Data were recorded on day 6.

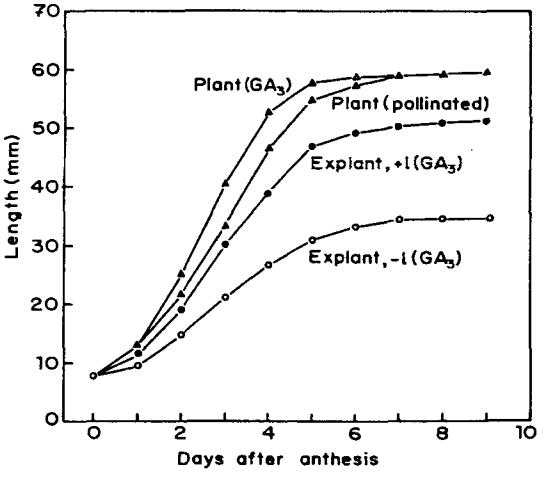

Fig. 6. Development of pea fruits on the intact plant (self-pollinated or unpollinated and GA_3-treated ovaries) and on explants with (+l) or without (−l) leaves. (GA_3) = 20 μl of 100 μg GA_3ml^{-1} per ovary. Explants without leaves were cultured into 50 mM sucrose, and explants with leaves were cultured in water. Each point represents the mean of four replicates.

centration (Fig. 5). The length of fruits developed from ovaries treated with 100 μg GA_3 ml^{-1} in explants with leaves cultured in a medium with sucrose was similar to that of fruits developed on the intact plant.

The pattern of development of the fruits on the explants was similar to the pattern of development of fruits on the intact plant and in both cases followed a sigmoid curve (Fig. 6). The final size of the pods in explants without leaves was significantly smaller, whereas the development of the ovaries on explants with leaves cultured in the absence of sucrose approached that of the attached fruits (Fig. 6). All the pods had a similar shape.

The growth of pea ovaries in the presence of sucrose alone was decreased by a 24 h preincubation in water, and even more by 3×10^{-5} M AMO-1618

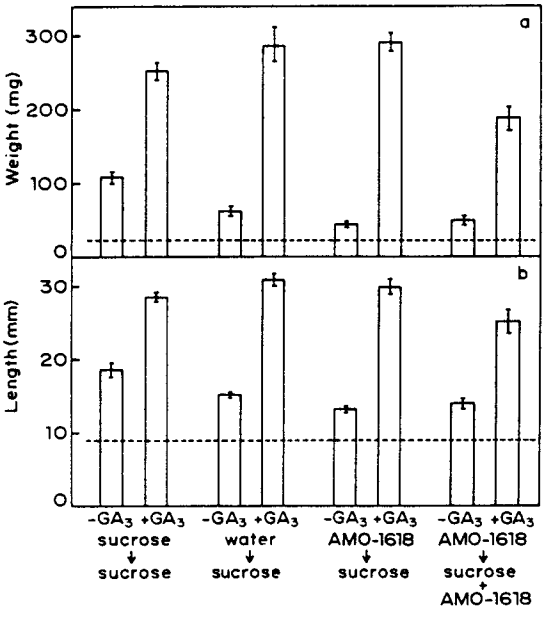

Fig. 7. Effect of AMO-1618 on the length (a) and weight (b) of parthenocarpic pea fruits. Explants were made on day 0, incubated for 24 h in the first solution indicated, then transferred to the second solution for 5 days. Data were recorded on day 6. Values are the mean ± SEM of two experiments, four replicates per experiment. Dotted lines represent the weight and length of the ovaries at day 0. $+GA_3$ = 20 µl of 100 µg GA_3ml^{-1} per ovary on day 1; sucrose = 100 mM sucrose; AMO-1618 = 3×10^{-5} M AMO-1618 in the vial.

(Fig. 7). This inhibition was not observed when the ovaries were treated with GA_3. Continuous feeding with 3×10^{-5} M AMO-1618 did not ellicit additional inhibition in unstimulated ovaries and prevented maximal development of the ovaries treated with GA_3 (Fig. 7).

Discussion

The results described in this paper show that pea ovary explants are an advantageous system for studying the hormonal and nutritional factors involved in fruit set and development. The use of explants retaining a portion of stem and of a liquid medium permits easy manipulation of the explants compared with explants using a small piece of pedicel and agar solidified medium (Nitsch 1951), and the incubation does not need to be carried out under rigorously sterile conditions. Nevertheless the addition of antibiotics, such as penicillin and chloramphenicol, which have been shown to be effective in controlling bacterial contamination without affecting growth of lentil seedlings *in vitro* (Seitz and Lang 1968), might be helpful when more complex nutrient media are used or when one would avoid periodically changing the solutions.

Interesting aspects of the pea ovary explant system are the capacity of the explants to respond to GA_3 for at least 1 day after excision (Fig. 2) and the rapid growth of the parthenocarpic fruit (Figs. 2 and 6), which attained its maximum size (about a 4-fold increase of length and a 22-fold increase of fresh weight) after 6 days of incubation. This result is in contrast with the relatively low rate of growth of different kinds of ovaries when smaller explants and semisolid media are used (Nitsch 1951, Peterson 1974, Wittenbach and Bu-

kovac 1980). Srivastava et al. (1980) found that the final size (about 20 mm increase in length) of pollinated pea ovaries incubated in liquid medium was reached after 15 days of incubation. The use of leafy explants, which produce parthenocarpic fruits similar to those obtained in the intact plant (Fig. 6), may also be helpful in investigations on the role of the leaves in fruit set and development. Indeed the segment of the stem retained by the explant has a clear influence on the response of the ovary (Fig. 3); this effect is mediated probably through the mobilization of substrates.

Following Nitsch (1951), sucrose has been widely used as a nutrient to the culture media of ovary and fruit explant. Leopold and Scott (1952) found that many other organic and inorganic compounds increased fruit set of excised tomato ovaries too, although no data on development of fruits after setting were given. However, in all cases an exogenous hormone was also necessary for the unpollinated ovaries to grow (Nitsch 1951, Leopold and Scott 1952, Peterson 1974, Kano and Asahira 1978). In the case of pea, the induction of fruit set and development by sucrose alone were counteracted by AMO-1618 (Fig. 7), an inhibitor of gibberellin biosynthesis (Baldev et al. 1965, Dennis et al. 1965); this inhibitor had no effect in the presence of GA_3. In ovary explants GA_3 was the only hormone able to sustain parthenocarpic fruit development. These results indicate that the explants have a limited capacity of gibberellin biosynthesis, and that endogenous gibberellin may be involved in fruit set in pea. It has also been previously shown in whole plants that GA_3, but not auxins or cytokinins, was the only hormone able to induce parthenocarpic fruits morphologically similar to seeded fruits (García-Martínez and Carbonell 1980, Sponsel 1982, Vercher et al. 1984).

Gibberellin-mediated hydrolysis of stored substrates could play a significant role in the control of growth, and a correlation has been found between invertase activity and gibberellin-induced elongation in several systems (Seitz and Lang 1968, Kaufman et al. 1973, Jones 1973). However, in the case of the pea ovary explants, since GA_3 also stimulated ovary development when sucrose was substituted by glucose or fructose, the effect of GA_3 might not be at the invertase level. This observation agrees with the results of Walker and Hawker (1976), where invertase activity increased in pollinated, naphthyl acetic acid–treated, and nonpollinated ovaries of *Capsicuum annuum* and *Citrullus lanatus*, although sucrose was imported only into the first two kind of ovaries.

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