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Light, dark and growth regulator involvement in groundnut (*Arachis hypogaea* L.) pod development

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Abstract

Gynophore elongation and pod formation were studied in peanut plants (*Arachis hypogaea* L.) under light and dark conditions *in vivo*. The gynophores elongated until pod formation was initiated. Pod (3–20 mm length) development could be totally controlled by alternating dark (switched on) and light (switched off) conditions, repeatedly. Gynophore elongation responded conversely to light/dark conditions, compared to pods. In this study we aimed to correlate the light/dark effects with endogenous growth substances. The levels of endogenous growth substances were determined in the different stags of pod development. Gynophores shortly after penetration into the soil, 'white' gynophores, released twice the amount of ethylene as compared to the aerial green ones, or to gynophores bearing pods. Ethylene inhibitors had no effect on the percent of gynophores that developed pods, but affected pod size which were smaller compared to the control. A similar level of IAA was extracted from gynophore tips of green gynophores, 'white' gynophores and pods. ABA levels differed between the three stages and were highest in the green gynophores and lowest in the pods.

Abbreviations: ABA = abscisic acid; AOA = aminooxyacetic acid; ELISA = enzyme linked immunosorbent assay; Ethrel = 2-chloroethanephosphonic acid; GC = gas chromatography; HPLC = High Performance Liquid Chromatography; IAA = indole-3-acetic acid; NAA = naphthalene acetic acid; RIA = radioimmunoassay; STS = silver thiosulfhate; TIBA = 2,3,6-triiodobenzoic acid.

1. Introduction

The groundnut (*Arachis hypogaea* L.) is a leguminous crop, in which flowering, self pollination and fertilization occurs above ground. Following aerial proembryo formation, an intercalary meristem located at the base of the ovary becomes active. The dividing cells form a gynophore (peg), which elongates gravitropically. The gynophore carries the ovary at its tip and grows into the soil where pod development usually occurs [1]. Darwin [2] showed that the gynophore is not responding in a negative phototropic way but responds directly to gravity. Light stimulates gynophore elongation and inhibits embryo and pod growth. Darkness arrests gynophore elongation and promotes the development of the ovary into a pod [3, 4].

Several studies indicated that gynophore elongation and embryo development are controlled by growth regulators. According to Jacobs [5], auxin is apparently produced in the distal 10 mm of the elongation gynophores. Applying increasing concentrations of auxin to gynophores from which the ovary was excised, resulted in a progressive growth inhibition of the gynophore. Changes in NAA and kinetin ratio during gynophore development *in vitro* seem to affect gravitropic responses, elongation of the gynophore and pod formation [3]. NAA given to both intact and decapitated gynophores inhibited elongation. TIBA also prevented elongation. The anatomy and elongation rate

of kinetin-treated intact gynophores was similar to the control [4]. Ziv and Kahana [6] who showed the inhibitory effect of both exogenous ABA and the presence of the ovular tissue on embryo growth, *in vitro*, suggested that light-induced the produce of ABA in the ovule, which is involved in the photo-inhibition of embryo development.

Although flowering of peanut plants was readily regulated by ethrel treatment, it had either no effect, or its effects were deleterious to yield and quality of peanuts over a wide range of concentrations and time of application [7].

The purpose of this study was to investigate the involvement of light and darkness in the cessation and reactivation of embryo and pod development, and to correlate between the levels of endogenous growth substances and developmental changes.

2. Materials and methods

2.1 *Plant material*

Peanut seeds (cv. Hanoch, supplied by Dr. I. Wallerstein, ARO, the Volcani Center, Bet Dagan, Israel) were sown in the field and grown in the summer (April–September), at the Faculty of Agriculture, Rehovot. During the winter months (October–February), the plants were grown in the Phytotron [8] of the Faculty of Agriculture, Rehovot. The seeds were sown in pots, in a 1:1 mixture of basalt gravel and vermiculite, at a 27 ± 1 °C day and 22 ± 1 °C night temperature, and 16 h photoperiod. The natural photoperiod was extended by supplementary illumination providing 104 erg.cm^{-2}.s^{-1}, at plant level. Gynophores were collected routinely from these plants.

Ovaries and pod development were examined at four stages. Green gynophores – aerial gynophores bearing ovaries, grown in the light; white gynophores, soil grown, shortly after penetration (tissue developed in the dark lacked chlorophyll); stage I (3–8 mm long); and stage II (10–20 mm long) pods that were developed in the soil.

2.2 *Intermittent light and dark conditions,* in vivo

In field grown plants under natural daylength and temperature, white gynophores, stage I and II pods were reexposed to light after developing underground. Gynophores from the four stages were kept, for 7 days above ground, in transparent glass vials containing a small amount of wet cotton-wool. The gynophores were then removed from the glass vials and were reintroduced into the soil, for 10 additional days (10–20 gynophores per treatment). Gynophore length and the pod long axis was measured before and after each treatment period.

2.3 *Endogenous growth substances*

Endogenous growth substances were determined in the four stages of development. Samples of the gynophore segments (0.5–1.0 cm) contained the ovary, the intercalary meristem and the gynophore tissue adjacent to it.

Measurements of ethylene released by excised green gynophores, white gynophores or stage I pods were carried out in glass vials (5 ml), sealed with rubber stoppers, kept at room temperature (25 ± 2 °). Vials containing the white gynophores and the pods were covered with aluminum foil. Each treatment consisted of 6–10 gynophores and was repeated 5 times. 2 ml air sample was drawn, with a plastic syringe, 30 min after excision. The stoppers were removed, the vials were ventilated, covered, and a second sample was taken after additional 60 min. The samples were injected into a GC (Vega series 2, Gas Chromatographs Carlo Erba Strumentazione). The amount of ethylene was calculated by comparing with known ethylene standards. The effects of ethylene inhibitors were examined *in vitro*. Tips of aerial gynophores (3–5 cm long) were excised, sterilized in 0.5–0.75% sodium hypochlorite + 0.01% Tween 10, for 15 min, and rinsed three times in sterile distilled water under aseptic conditions. Explants were trimmed to 10–15 mm before planting, and were cultured in a modified Murashige and Skoog [9] medium with 0.5 μM kinetin and 0.3 μM NAA. Various concentrations of AOA or STS were added to the medium. The vials (25 × 100 mm) containing 10 ml medium and 1–2 gynophores were wrapped in black plastic bags. Each treatment consisted of 14–40 replicates and was repeated at least twice. The cultures were kept for 26–30 days, at 25 ± 1 °C.

IAA and ABA levels were determined by two methods. The radioimmunoassay (RIA) was performed as described by Bohner and Bangerth [10], with slight modifications, detailed here. Four samples of 100 mg freeze-dried tissues per treatment were extracted and dissolved in 0.1 M phosphate buffer (pH 8). After centrifugation the supernatant was passed through a polyvinyl-polypyrolidone (PVPP) column, and eluted with phosphate buffer. The extract was

Table 1. Changes in gynophore length after light period (7 days) followed by dark period (10 days)

Gynophore stage (when experiment started)	Increase of gynophore length (cm)	
	7 days in light	10 days in dark
green gynophores	3.5 ± 0.7*	2.7 ± 0.3*
white gynophores	0.8 ± 0.3	0.9 ± 0.4
gynophore bearing stage I pods	0.0 ± 0.0	0.0 ± 0.0
gynophore bearing stage II pods	0.0 ± 0.0	0.0 ± 0.0

* mean ± SE.

adjusted to pH 2.5–3.0 passed through a C_{18} Sep-Pak cartridge as described. The following steps were also the same as described by Bohner and Bangerth [10].

The determination of IAA by the ELISA and ABA by the GC method were carried out according to Sagee *et al.* [11], with slight modifications that are detailed here. Four samples of 100 mg freeze-dried tissue per treatment were used. Internal standards of 182 pmol ^{14}C IAA (2GBq mmol^{-1}) and 0.4 pmol ^{3}H ABA (925GBq mmol^{-1}) were added to each sample. After methylation the extracts were evaporated to dryness with N_2 and redissolved in 0.75 ml absolute methanol. Each sample was passed through an HPLC (Packard, Hp 1090, set at 254 nm), RP-18 column (5 μ, 15 cm, Merck). Elution was performed with a 52 min gradient of 10–60% methanol in water. Both solvents contained 1% (v/v) acetic acid. The flow rate was 0.6 ml.min^{-1}. The fractions containing IAA and ABA were collected and evaporated to a small volume and then dried under a stream of nitrogen.

The extracts, containing ABA, were redissovled in 0.2 ml of toluene. Samples of 2 μl were injected to a Mega Bore BB17 column, 15 m length, diameter 1 μ (J&W Scientific), 3300 Varian GC. The entrance temperature was 250 °C, column – 220 °, detector – 300 °C, N_2 flow – 10 ml.min^{-1}. The amount of ABA was calculated by comparing to standards of cis-trans synthetic methylated ABA. The results indicate that the extracts even after cleaning on a HPLC column, contain substances other than the hormones, that react like the hormones. Therefore, in the results we will relate only to the relative levels of the growth substances. Results are given as mean ± SE (The means are shown by histograms; the bars indicate SE.).

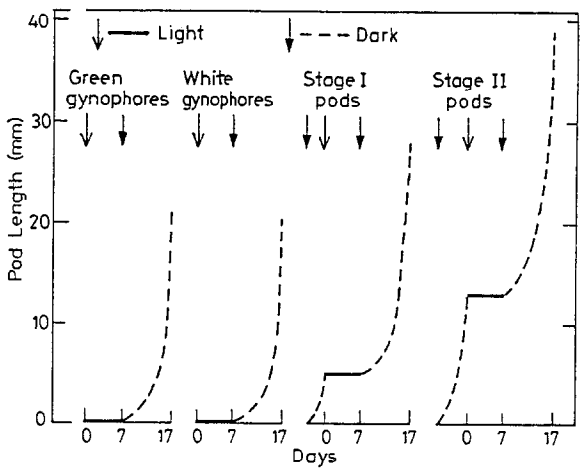

Fig. 1. The effect of light and dark treatments on pod length, at different developmental stages *in vivo*. Broken lines represent the rate of elongation during the dark period (the broken lines were extrapolated by the initial and final values of pod length, assuming that at the beginning of the dark period the elongation is slower than later). Horizontal lines represent inhibition of pod elongation during the light period.

3. Results and discussion

3.1 *The effects of light and dark on gynophore and pod development*

Aerial green gynophores elongating in the light, continued to elongate for some time after being transferred to the dark (Table 1). White gynophores continued to elongate when re-exposed to light, or when introduced back into the soil, though to a lesser extent compared to the green gynophores. No elongation was observed in gynophores possessing stage I or stage II pods when exposed to light, or reintroduced into the soil. The effect of light and dark treatments on pod length is illustrated in Fig. 1. White gynophores exposed to light and then reintroduced into the soil, developed pods equal in size to green gynophores that were introduced into the soil. Interruption of *in vivo* dark grown pods by light, was followed by complete cessation of pod growth, but when reintroduced into the soil their development continued.

The intercalary meristem, located at the base of the ovary, causes the gynophore to elongate in the light [1, 4, 12, 13]. The meristematic activity in the intercalary meristem is gradually diminished. The gynophore ceases to elongate, once a pod has started to develop. Moss *et al.* [14] and Pattee *et al.* [13] showed that, when the intercalary meristem remains attached

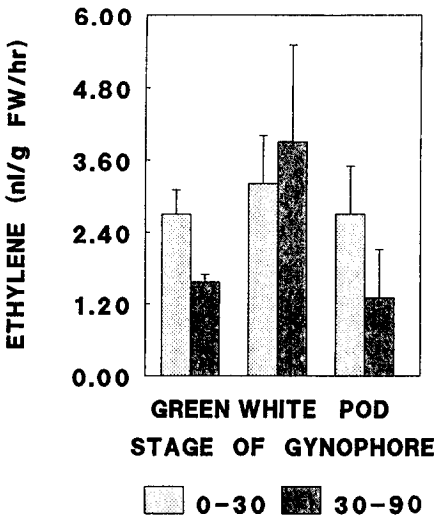

Fig. 2. The production of ethylene by green gynophores, white gynophores and stage I pods, 30 min after excision and 30–90 min after excision.

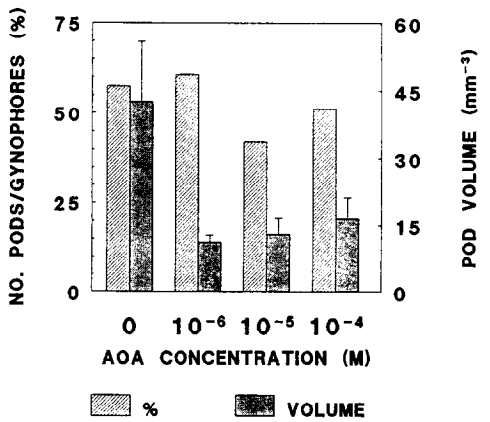

Fig. 3. The effect of AOA on the number and volume of developing pods.

to gynophore tips grown *in vitro*, ovular growth is suppressed. It seems that the embryo can resume growth and the pod to develop only after the meristematic activity is arrested. Light was found to enhance gynophore elongation and to inhibit pod formation *in vitro* [3]. Narasimhulu and Reddy [15], also reported that interruption of dark-grown cultures with light lead to complete cessation in growth of the pod. In this report we show that pod formation could be switched on (by dark) and off (by light) repeatedly, at any developmental stage (3–20 mm), but the cessation of cell division in the intercalary meristem, upon dark conditions, is irreversible.

Growth of the peanut embryos, when removed from the influence exerted by ovular tissue, was unaffected by the light [16]. Light, however, inhibited embryos left intact in halved ovules [6]. One of the ways which the ovule mediates photocontrol over embryo growth could be through the production of growth regulators which diffuse to the embryo.

3.2 *Endogenous growth substances*

3.2.1 *Ethylene*

Following excision; green gynophores, white gynophores or stage I pods, released similar amounts of ethylene during the first 30 min after excision (Fig. 2). During the following 60 min, less ethylene was released from green gynophores and from stage I pods (we are aware to the great variability but, the same trend was found also in a repeated experiment). We assume that the first 30 min, represent a wound response [17], while ethylene release during the next 60 min, represents the changes which occur *in vivo*. During 30–90 min, the white gynophores released twice the amount of ethylene as compared to the green gynophores or to stage I pods. This can be explained, as a result of friction with the soil during penetration, *in vivo*. Qi-li and Rui-chi [18] reported that before the ovary penetrated into the soil ethylene evolution was less than 15 nl $g^{-1}FW^{-1}$; 1–3 days after the gynophore penetrated the soil, the ovary not yet enlarged, ethylene increased threefold; during ovary enlargement, the content of the ethylene first increased then decreased. Conversely, Hedges and Fletcher [19], reported that gynophores that had not penetrated the soil evolve approx 7 nl g^{-1} h^{-1}; gynophores which had just penetrated the soil 9.5 nl g^{-1} h^{-1}; and older gynophores showed a decrease to 3.5 nl g^{-1} h^{-1}. These results are in concomitance with our results. In our work we related to ethylene evolution from a defined morphological stage.

AOA is a potent inhibitor of ethylene production. AOA prevent the conversion of S-adenosylmethionine (SAM) to the ethylene precursor aminocyclopropane carboxylic acid (ACC) [20]. AOA ($10^{-6} - 10^{-4}M$) had not effect on the percent of gynophores that developed pods (Fig. 3), but those pods that developed were smaller compared to the control (Fig. 3). Silver ions have been shown to be a potent ethylene antagonist [21]. Silver thiosulfhate (STS, $10^{-6} - 10^{-4}MM$) had similar effect as that of AOA. As the concentration increased, smaller pods developed, indicating a dose response (Fig. 4).

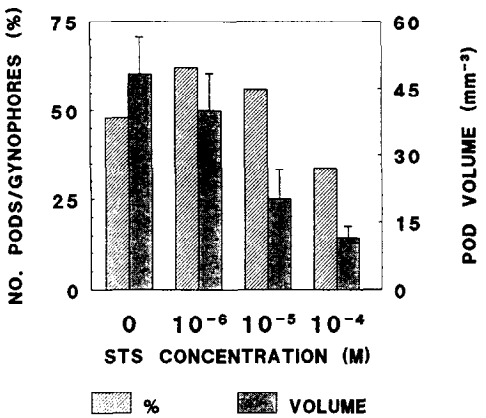

Fig. 4. The effect of STS on the number and volume of developing pods.

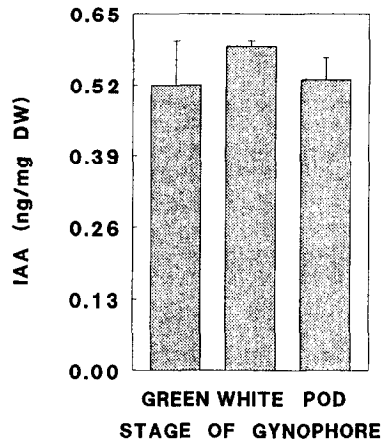

Fig. 5. IAA levels in green gynophores, white gynophores and stage I pods, obtained by the ELISA method.

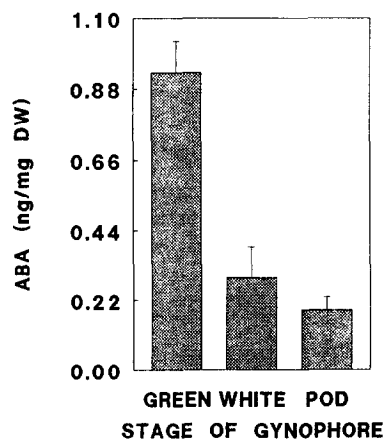

Fig. 6. ABA levels in green gynophores, white gynophores and stage I pods, obtained by the GC method.

Ethylene inhibitors, both AOA and STS, had no effect on the percent of gynophores that developed pods, this implies that ethylene is not involved in removing the restriction on embryo development caused by light. This assumption get support from filed experiments; ethrel treatment had either no effect or its effects were deleterious to yield [7]. Since the inhibitors affected pod's size, it is possible that ethylene may have a role in affecting growth and determining pods size. The changes that occur in the gynophore after soil penetration (The part of the gynophore which develop in the soil lake chlorophyll) resemble the 'triple response' of etiolated pea seedlings to ethylene; inhibited stem elongation, radial stem swelling and a horizontal growth habit [22]. This coincides with the finding of high ethylene secretion from the white gynophores.

3.2.2 *IAA*

The difference between IAA level, expressed on dry weight basis, in the green gynophores, white gynophores and stage I pods, obtained by the ELISA assay (Fig. 5), were small. IAA determined by the RIA method showed similar results. Therefore, it can be concluded that the IAA level is similar in the green, white gynophores and in stage I pods. Contrary to our results, Jacobs [5], Qi-li and Rui-chi [18] reported that there was an increase in the amount of auxin in the enlarging ovary. Kahana [23], however, showed that the growth of globular and cotyledonary stage embryos was inhibited in a medium containing 0.1 $mg.l^{-1}$ IAA. In general, low auxin concentrations promote embryo development while high concentrations arrest embryo development [24]. Various developmental stages and diverse methods, that were used to identify and quantify endogenous growth substances, may be the cause for the contradictory results reported by different researchers. In spite of the contradictory results, auxin may play a regulatory role in gynophore elongation and pod development.

3.2.3 *ABA*

ABA levels, measured by the GC method, in green gynophores were 3 and 5 times higher than in the white gynophore and stage I pod, respectively (Fig. 6). Similar results were obtained by the RIA method. It is possible that the high levels of ABA found in the green gynophores are the cause for embryo arrest in the light. Exogenously applied ABA ($10^{-3}M$) inhibited

development of dark grown, excised embryos [6]. It is suggested, therefore, that high levels of ABA, found in the green gynophores under natural conditions in the light, cause embryo arrest. In the dark, endogenous ABA levels are reduced and embryo development can be resumed.

4. Concluding remarks

We have demonstrated that the cessation of cell division in the intercalary meristem, caused by dark, is irreversible. Pod development, on the contrary, could be switched on and off, by alternating dark and light conditions. Pod inhibition imposed by light may be linked to high ABA levels found in the green gynophore. Lower levels of ABA were found in the ovaries which started to develop in the dark. Therefore, it can be assumed that when the gynophore is exposed to light, ABA levels increase and in the dark they decline. Ethylene inhibitors had no effect on the percent of gynophores that developed pods, and IAA levels were similar in the different stages, suggesting that both ethylene and IAA are not involved in removing the restriction, caused by the light, on embryo and pod development.

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