Ethylene production as an indicator of chilling injury in oil palm (*Elaeis guineensis* Jacq.) somatic embryos

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Oil palm (*Elaeis guineensis* Jacq.) somatic embryos are very susceptible to chilling injury. The critical temperature is found to be close to 18°C. The development of injury increases with decreasing temperature and with extending exposure to chilling temperatures. Ethylene production is inhibited at temperatures below 18°C, however it is enhanced after transfer of somatic embryos to warmer temperature (27°C). This chilling induced ethylene production decreases with extending chilling treatment. Results obtained suggest that ethylene release after warming chilled tissues could be a good indicator of chilling injury severity. Repairing of damage induced by chilling temperatures is associated with an increased activity of the enzymatic system (ethylene forming enzyme) which converts ACC to ethylene.

Key words: oil palm; *Elaeis guineensis*; somatic embryos; chilling injury; ethylene; ethylene-forming enzyme

Introduction

Many species of tropical and subtropical origin are injured or even killed by extended exposure to low non-freezing temperatures. The physiological damage to plant tissues which could give rise to death are commonly referred to as 'chilling injury' [1,2]. The critical temperature, in the range of 20°C down to about 0°C, for chilling injury has been reported to be a characteristic of the species, but it depends on developmental stages, organs and tissues [1,2]. The disorders increase with degrees of chilling, however a feature of this phenomenon is generally its reversibility following short exposure to low temperatures [1,2].

The production of ethylene by plant organs or tissues is widely used as an indicator of stresses [3—5]. Ethylene release is temperature-dependent and is generally reduced at low temperatures [6]. However, chilling exposure can enhance ethylene production of chilling-sensitive plant tissues [6—12]. Depending on the tissues or the species the increase in ethylene release occurs during the chilling treatment or only after transfer to warmer temperatures [10].

In other respects, as ethylene is involved in plant growth [3], the production of this hormone might be a good indicator of the recovery of growing tissues after chilling exposure.

The aims of the present work were (i) to investigate the effects of chilling temperatures on the growth of oil palm (*Elaeis guineensis* Jacq.) somatic embryos and (ii) to search for a relationship between chilling injury and ethylene production during exposure at relatively low temperatures or after transfer to warmer temperature. Oil palm was chosen because this species is very chilling sensitive [13], and somatic embryos were used since they are easy to produce and to manipulate.

Materials and Methods

Production and culture of somatic embryos

Somatic embryos were produced from the clone BC 068 of oil palm, and were obtained according

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to a method developed by the ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération)/IRHO (Institut de Recherches pour les Huiles et Oléagineux) research team [14] in Bondy, France. The embryoid clumps used were cultured on a standard medium corresponding to the Murashige and Skoog medium devoid of growth regulators and containing 0.1 M sucrose [15]. Each clump comprised adventitious embryos of different sizes and at different developmental stages, which were linked together. Cultures were performed in 37-ml tubes (one embryoid clump per tube). The average weight of embryoid clumps was around 500 mg at the beginning of experiments.

Embryoid clumps were placed in darkness at 10°, 12°, 15°, 18° and 20°C for various durations and then transferred to 27°C, the optimal temperature for their growth [16]. Control cultures were maintained continuously at 27°C.

Growth measurements

Each embryoid clump was weighed at the beginning of experiments and after 6 weeks of culture at 10°, 12°, 15°, 18°, 20° and 27°C. Embryoid clumps that had grown were then divided into parts of about 500 mg. After weighing of each part all embryoid clumps were transferred to 27°C and weighed again 6 weeks later. The increase in weight after the first 6 weeks of culture represented the ability to grow at the different temperatures, whereas weight increase during the second period of 6-week culture quantified the potential recovery of growth at 27°C following the treatment at lower temperatures. Results were expressed as % of weight increase of each embryoid clump, and correspond to the mean of 12 measurements ± S.D.

Survival of embryoid clumps was evaluated by the number of clumps among a population of 12, which were able to grow at 27°C after various periods of culture at lower temperatures.

Measurement of ethylene production

Ethylene production was measured with embryoid clumps placed at 12°, 18° and 27°C, or at 27°C after various durations at 12° and 18°C. For each measurement the tubes were tightly closed for 24 h and 1 ml of gas sample was taken with a syringe and injected in a gas chromatograph (type 330, Girdel, France) using a flame ionisation detector, through an activated alumine column (6 mm in internal diameter, 50 cm long, 50—80 mesh). The carrying gas was nitrogen and the column temperature was 60°C. The minimal detectable ethylene quantity was 0.01 nl. Results were expressed as nl of ethylene produced by one embryoid clump in 24 h, and correspond to the mean of 12 measurements ± S.D.

Treatment with 1-aminocyclopropane 1-carboxylic acid

To determine the capability of somatic embryos to produce ethylene from 1-aminocyclopropane 1-carboxylic acid (ACC), embryoid clumps were first cultured for 6 weeks at 12° and 18°C on the standard medium. They were then soaked for 1 h in a solution of 1 mM ACC and transferred to 27°C on the standard medium containing 1 mM ACC. Ethylene production was measured as previously described, using 12 embryoid clumps, and was compared to ethylene production in the absence of exogenous ACC. Measurements were performed during 14 days after transfer to 27°C. Control embryoid clumps were placed directly at 27°C.

Results

Effects of temperature on growth

The growth of embryoid clumps was very sensitive to temperature (Fig. 1). It decreased sharply from 27° to 18°C, and was almost nil at lower temperatures.

Moreover, a 6-week culture at temperatures lower than 18°C had a strong inhibitory effect on further growth of embryoid clumps after transfer to 27°C (Fig. 2).

Survival of embryoid clumps, evaluated by their ability to grow when transferred to 27°C, depended on the temperature and the duration of previous culture (Table I). All clumps were dead after 6 weeks at 10°C, and after 30 weeks at 12°C. A culture longer than 12 weeks at 18°C also induced the death of some clumps, but all clumps remained viable at 20°C even after 30 weeks.
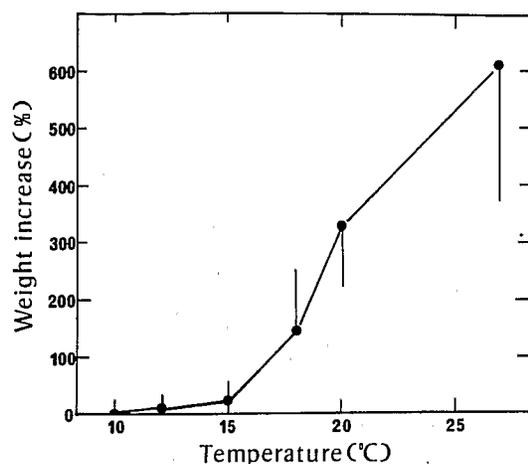
Fig. 1. Variation with temperature, of weight increase (in %
of initial weight) of embryoid clumps after 6 weeks of culture.
Mean of 12 measurements ± S.D.

Clumps which did not grow when transferred to
27°C, and which were then considered as dead,
became progressively brown presumably because
of oxidation of phenolic compounds.

Effects of temperature on ethylene production

Table II shows the production of ethylene by
embryoid clumps cultured at 12°, 18° and 27°C.
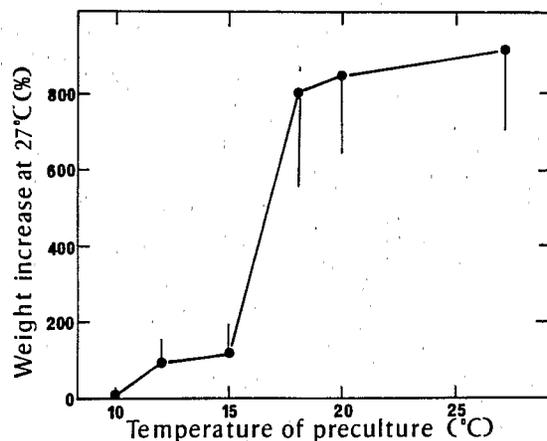Ethylene production was relatively high at 27°C,

Fig. 2. Variation with temperature of a 6-week preculture,
of weight increase of embryoid clumps placed at 27°C for 6
weeks (in % of the weight at the moment of transfer to 27°C).
Mean of 12 measurements ± S.D.

Table I. Number of surviving embryoid clumps, among
populations of 12 clumps, after culture for 6—30 weeks at 10°,
12°, 15°, 18° and 20°C. Survival was evaluated by the ability
of clumps to grow when transferred to 27°C.

Temper-ature of preculture (°C)	No. of surviving clumps after preculture for (weeks)				
	6	12	18	24	30
10	0	0	0	0	0
12	9	7	4	3	0
15	12	9	8	7	4
18	12	12	11	10	10
20	12	12	12	12	12

and it increased with duration of culture probably
because of growth of embryoid clumps. At 18°C,
ethylene production was much lower and
remained almost at the same value (20—30 nl/day
by one embryoid clump) during the 11 weeks of
experiment though embryoid clumps were able to
grow slowly (cf. Fig. 1). At 12°C, the embryoid
clumps lost progressively their ability to produce
ethylene.

Changes in ethylene production by embryoid
clumps placed at 27°C after different durations of
culture at 12°C and 18°C, are shown in Fig. 3. A
burst of ethylene appeared after transfer of
embryoid clumps from 18° to 27°C (Fig. 3A). The
increase in ethylene release occurred within the
first week at 27°C. It was very marked after 2—6

Table II. Variation of ethylene production (nl/24 h by one
embryoid clump) by embryoid clumps during their culture at
12°, 18° and 27°C. Mean of 12 measurements ± S.D.

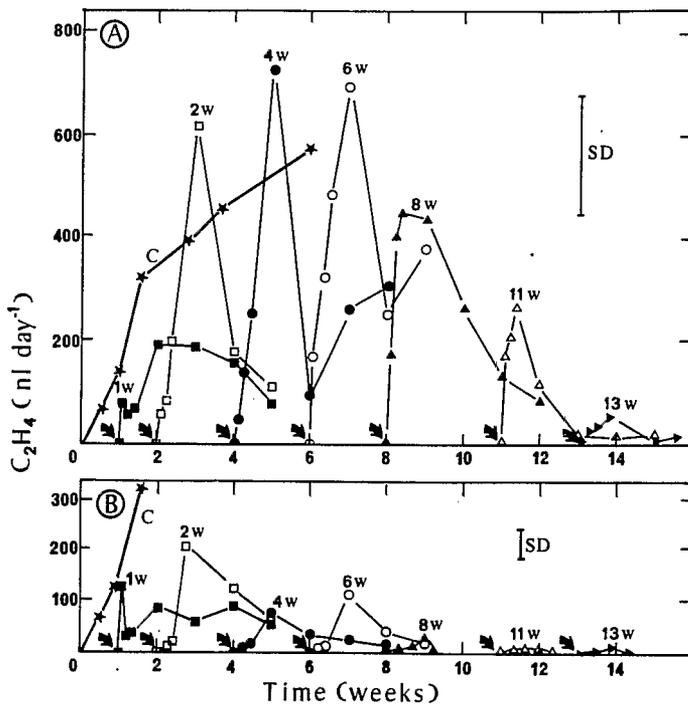| Duration of culture (weeks)	Ethylene production (nl/24 h/embryoid clump) at		
	12°C	18°C	27°C
1	20.2 ± 7.3	28.2 ± 7.4	365.9 ± 39.2
2	3.7 ± 3.8	23.7 ± 7.9	644.2 ± 50.3
4	1.1 ± 0.4	19.5 ± 6.2	702.7 ± 78.3
6	0.7 ± 0.4	19.1 ± 8.1	868.4 ± 103.3
8	0.4 ± 0.3	30.6 ± 9.2	—
11	0.4 ± 0.4	32.8 ± 10.6	—

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Fig. 3. Variation during culture at 27 °C, of ethylene production (nl/24 h by one embryoid clump) by embryoid clumps previously placed for 1, 2, 4, 6, 8, 11 and 13 weeks at 18 °C (A) or 12 °C (B). Control unchilled embryoid clumps (C) were placed directly at 27 °C. Mean of 12 measurements. Vertical bars denote the largest S.D. Arrows indicate at which times the embryoid clumps were transferred from 18° or 12 °C to 27 °C. The data points below the arrows represent ethylene production before transfer to 27 °C.

weeks of culture at 18 °C, and was then reduced when exposure to 18 °C was prolonged. The same phenomenon was observed with embryoid clumps transferred from 12° to 27 °C (Fig. 3B). However, the burst of ethylene was much less intense and practically disappeared after 6 weeks of culture at 12 °C.

Effects of temperature on ACC conversion to ethylene

Figure 4 shows the development of ethylene production at 27 °C in the presence of 1 mM ACC, of control embryoid clumps and of embryoid clumps previously cultured for 6 weeks at 12° and 18 °C. Control embryoid clumps placed directly at 27 °C produced much more ethylene in the presence of ACC than in the absence of this compound (compare with Table II), and they converted more and more ACC during culture. Embryoid clumps which were precultured at 18 °C
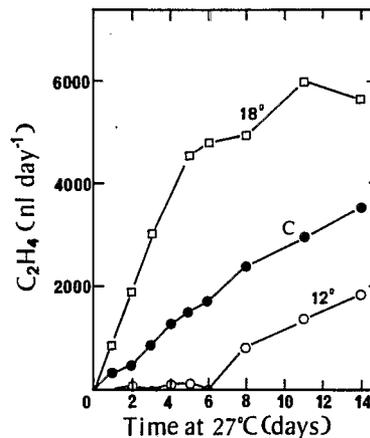

Fig. 4. Variation during culture at 27 °C in the presence of 1 mM ACC, of ethylene production (nl/24 h by one embryoid clump) by embryoid clumps previously placed for 6 weeks at 12° or 18 °C. Control embryoid clumps (C) were placed directly at 27 °C in the presence of ACC. Mean of 12 measurements ± S.D.
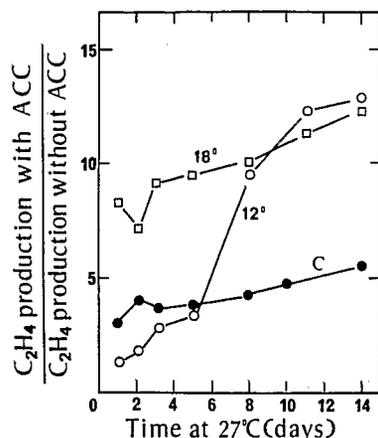
Fig. 5. Variation during culture at 27°C, of the stimulation of ethylene production by 1 mM ACC (expressed as the ratio of ethylene production in the presence of ACC to ethylene production in the absence of ACC) for embryoid clumps previously placed for 6 weeks at 12° or 18°C. Control embryoid clumps (C) were placed directly at 27°C. The ratio was calculated from mean values of 12 measurements.

produced more ethylene from ACC than the control ones. This production of ethylene occurred immediately after transfer to 27°C, and became maximal after about 1 week. Production of ethylene by embryoid clumps previously placed at 12°C was very low during the first week at 27°C. It increased afterwards, but remained lower than with control embryoid clumps.

The stimulation of ethylene production by ACC, quantified by the ratio of ethylene production in the presence of ACC to ethylene production in the absence of ACC, is shown in Fig. 5. The value of this ratio was around 4—5 for control embryoid clumps placed directly at 27°C, and reached 9—11 for embryoid clumps previously placed at 18°C. In the case of embryoid clumps precultured at 12°C the effect of ACC on ethylene production was weak during the first 5 days of culture at 27°C, and then increased sharply.

Discussion and Conclusion

Like many organs of tropical and subtropical plants [1,2,13], Elaeis guineensis somatic embryos are very susceptible to chilling injury. A minimal

temperature for growth (Fig. 1) and survival (Fig. 2 and Table I) of embryoid clumps has been found to exist around 18°C. Similar results were reported by Mok and Hor [17] for the seeds of the same species. The severity of injury increases with decreasing temperature and with extending exposure to chilling temperatures (Table I).

Production of ethylene by oil palm embryoid clumps is high at 27°C, whereas it remains low at 18°C and decreases markedly at 12°C (Table II). However, it is strongly enhanced after transfer from 18° to 27°C (Fig. 3A). Such an enhanced release of ethylene is also observed, though at a lesser extent, with embryoid clumps transferred from 12° to 27°C (Fig. 3B). A burst of ethylene production after transfer to warmer temperature has been observed with various organs of chilling sensitive plants such as Citrus [7], Cucumis sativus [11,18,19], Carica papaya [12] and Phaseolus vulgaris [19]. In oil palm somatic embryos, this enhanced ethylene synthesis decreases with extending chilling treatment (Fig. 3), and is suppressed faster for somatic embryos exposed to 12°C (Fig. 3B) than for those exposed to 18°C (Fig. 3A). It seems, therefore, that the ability of somatic embryos to produce ethylene at 27°C reflects the severity of damage induced by chilling temperatures, and the possible recovery of the cultures after chilling stress. These results suggest that, as previously shown by Chen and Patterson [20], the release of ethylene after warming chilled tissues could be accepted to be a good indicator of chilling severity. A burst of ethylene after transfer to warm temperature is indicative of weak chilling stress, whereas a too severe stress induces an inability to produce ethylene.

In higher plants, ethylene is mainly synthesized through the pathway from S-adenosylmethionine (SAM) to ACC and from ACC to ethylene [4], and the enzymatic system ethylene-forming-enzyme (EFE) which converts ACC to ethylene has been shown to be associated with cell membranes [4]. The results obtained by applying exogenous ACC to oil palm somatic embryos show that EFE is affected by chilling temperatures. EFE is activated after treatment for 6 weeks at 18°C, since chilled embryos transferred to 27°C produce more ethylene than control unchilled ones, in the

presence of ACC (Fig. 4). Moreover, the stimulation of ethylene production by ACC is higher in embryos precultured at 18°C than in embryos placed directly at 27°C (Fig. 5). Exposure for 6 weeks at 12°C results in a marked reduction of ACC conversion to ethylene during the first days following transfer to 27°C (Fig. 4). Such an effect of too low a temperature, which was observed with various chilling sensitive materials [11,18,20], suggests that the EFE system is impaired, probably because chilling affects membrane integrity or lipid-protein composition of membranes [1,21] However, oil palm somatic embryos precultured for 6 weeks at 12°C recover the ability to convert ACC to ethylene when maintained for more than 5 days at 27°C (Fig. 4), and this recovery is associated with an activation of EFE similar to that observed with embryos prechilled at 18°C (Fig. 5). It seems, therefore, that repairing of damage induced by chilling temperatures is accompanied with an increased activity of EFE.

Precise analyses of EFE activity in relation to membrane properties and composition during chilling and recovery processes are the subject of further studies.

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