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Control of *Dioscorea alata* microtuber dormancy and germination by jasmonic acid

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Abstract

Effects of appling exogenous jasmonic acid (JA) on the germination of *Dioscorea alata* L. microtubers were examined on Murashige and Skoog (MS) medium. Microtuber germination was promoted by JA (0.1 and 1 μM) supplemented to the culture medium but higher concentrations (30 and 100 μM) completely inhibited germination. When these inhibited microtubers were transferred to hormone-free medium, germination resumed.

After transfer to greenhouse conditions, almost all plants (95%) from tubers previously cultivated on MS medium with 100 μM JA survived and all acclimatized plants had produced tubers after 8 months. It is concluded that depending on JA concentration, both the germination and dormancy processes in *D. alata* microtubers were affected. The release from dormancy is easily obtained by transferring dormant microtubers to hormone-free medium.

Keywords : dormancy ; germination ; jasmonic acid ; microtubers ; storage ; yam

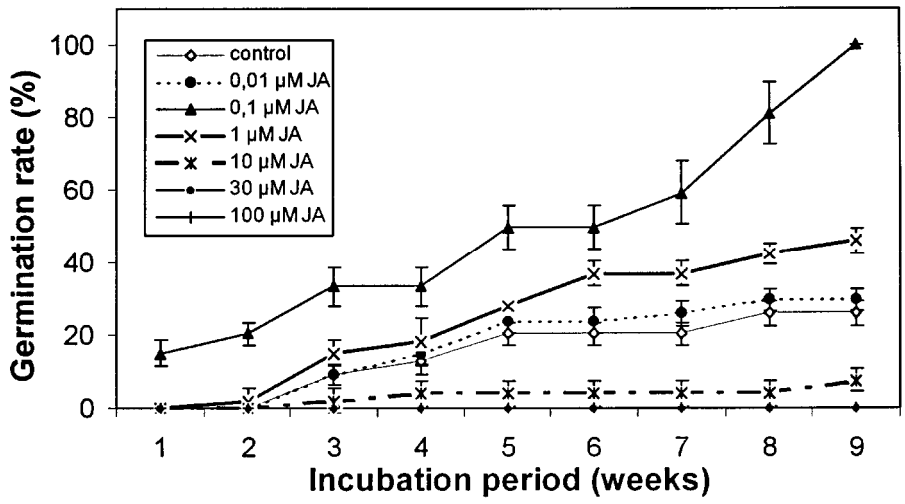
Abbreviations : JA : jasmonic acid ; MS : Murashige and Skoog medium

1. Introduction

The genus *Dioscorea* (yams) contains over 600 species [9, 11], some of which are important for humans as nutrient and pharmaceutical resources. Tubers from specific yam species are used in pharmacy as a source of diosgenin derivatives [2, 4]. The edible species are important food crops in many tropical countries especially in West Africa, South Asia and the Caribbean [9, 18]. The food species of yams do not produce viable seeds, with the exception of *D. trifida,* and *D. cayenensis-rotundata* [1]. Thus, yam is propagated vegetatively, generally using fragments of tubers or bulbils [8, 19]. Yams have a seasonal production, so that storage is necessary before planting and use as food. After harvest, tubers enter into dormancy, which is of maj or importance in yam storage because it determines the length of the storage period [17]. Once sprouting occurs, storage is no longer possible.

One of the major problems in yam is to find simple methods to prolong the dormancy period of tubers and to break this dormancy when required. Low temperatures (15 °C) together with fungicide treatment, or ionising radiation (0.08-0.12 KGγ) have been reported to prolong the storage duration in yam [3]. Passam [15] reported a lack of success in prolonging dormancy effectively by the application of sprout-suppressant chemicals such as those widely used for potatoes. A likely reason for this is that in yam, sprouts are not formed until a late stage of dormancy and they originate from beneath the periderm, thus being protected from the effects of such treatment. A more promising approach to prolong dormancy and hence to increase storage life is by the application of plant growth regulators. Gibberellic acid (GA3) is well known to induce dormancy in yam tubers [13, 14], but the break of dormancy in these GA3-treated tubers requires an additional treatment (i.e. cold temperatures). To our knowledge, GA3 does not promote yam tuber germination. In the work reported here, we studied the effect of jasmonic acid (JA) on dormancy and germination in *Dioscorea alata* L. microtubers. Since it has been reported that JA can induce tuberization in both yam and potato [6, 7, 16].

Figure 1. *Effect of jasmonic acid (JA) on germination of Dioscorea alata microtubers. Vertical bars represent standard errors (n = 3).*



2. Materials and methods

2.1 Plant materials

Microtubers of *Dioscorea alata* L. (*Dioscoreaceae*) were obtained from a private collection growing in greenhouses of the National Botanical Garden at Meise (Belgium) [Herbarium specimen number 5993]. Nodal stem segments of 1-2 cm in length were prepared, the lamina and petiole being removed. Expiants were immersed for 1-2 minutes in 80% ethanol (v/v), then for 30 minutes in 1% benomyl (w/v). After a distilled water wash, expiants were placed in a 3.5% (v/v) solution of sodium hypochlorite (commercial bleaching agent at 12° chloride, LA CROIX®) to which 0.01% of Tween 20 had been added. After 9 minutes, the expiants were washed four times with sterile distilled water. The cut ends of the expiants were trimmed aseptically and the basal ends cultured in hormone-free MS medium [10] supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar-agar. The microtubers obtained were then used for germination experiments. Cultures were incubated at 25±2 °C under 16 hours photoperiod. Light intensity was 30 μmol m^{-2} s^{-1}.

2.2 Microtuber germination experiment

The *D. alata* microtubers of more or less homogeneous size (2.5 cm length × 0.5 cm diameter) obtained were used as starting material. They were exposed for 7 h in a laminar flow clean bench for surface desiccation and then stored at 25±2 °C under 16 h photoperiod. After 2 weeks of storage, microtubers were incubated on basal MS medium supplemented with 3% sucrose (w/v), 0.5% (w/v) agar-agar and various concentrations of JA (0, 0.01, 0.1, 1, 10, 30 and 100 μM). Observations on germination were made once a week, over a period of 9 weeks. Germination was scored, when the length of the germinated shoot exceeded 5 mm.

2.3 Transfer of microtubers to JA-free MS medium

After 9 weeks of culture on the above mentioned media, microtubers which had not germinated were washed with distilled sterile water and maintained in water for 30 min. They were then transferred to the JA-free MS medium and incubated at 25±2 °C under a 16 h photoperiod. Observations on germination were made once a week over a period of 14 weeks.

2.4 Plant acclimatization and subsequent growth

All plants grown from microtubers cultured on medium containing 100 μM JA and on the JA-free MS medium, were transferred to a greenhouse for acclimatization during spring and summer. Roots were sectioned and the sections planted individually in pots (21 × 12 × 19 cm) filled with sand, compost and mould mixture (1:1:2). The pots were watered every day, during the first 2 months of the acclimatization, and 4 or 5 times per week, from the third month, during which time tubers developed and matured. The greenhouse culture conditions were on

average 24 °C and 50% of relative humidity.

Figure 2. *Dioscorea alata microtubers germination on hormone-free MS medium. Microtubers were precultivated in various concentrations of JA for 9 weeks. Vertical bars represent standard errors (n = 3).*

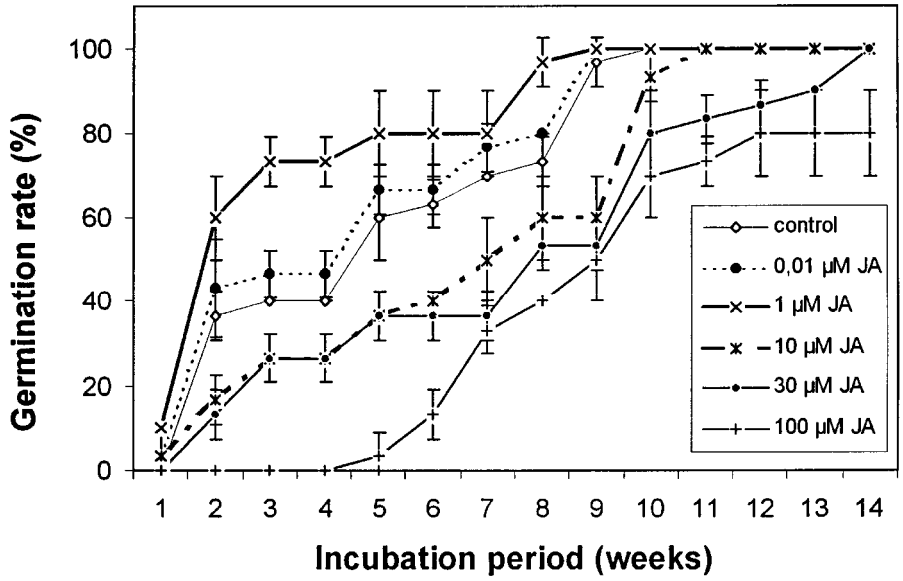
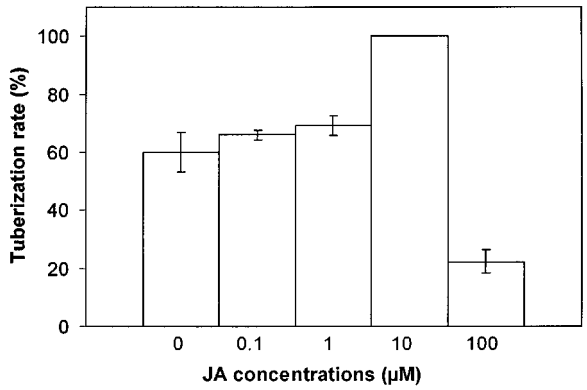


Figure 3. *Effect of Jasmonic acid on tuberization of Dioscorea alata after two months of culture. Vertical bars represent standard errors (n = 3).*



3. Results and discussion

The *D. alata* microtuber stock used in this study was obtained from *in vitro* culture of nodal segments in hormone-free MS medium (see materials and methods). Microtuber germination was affected by exogenous JA incorporated into the culture medium. Indeed, germination on JA-free medium commenced at week 3 (Figure 1) and reached 20% after 5 weeks of culture; the rate of germination was stable until week 9. After transfer of the non-germinated microtubers to fresh hormone-free MS medium, germination started after 2 weeks (40% germination) and reached 100% after 10 weeks (Figure 2). Among the different JA concentrations tested, 0.01 μM of JA has no significant effect on microtuber germination. The most effective concentration (0.1 μM of JA) resulted in 15% germination in the first week rising to 100% at week 9 (Figure 1). Conversely, the highest concentrations of JA (30 and 100 μM) completely inhibited microtuber germination. Interestingly, when these JA-inhibited microtubers were transferred to hormone-free medium, the germination resumed with some delay (especially for those microtubers removed from medium with 100 μM JA) (Figure 2). Ungermin-ated

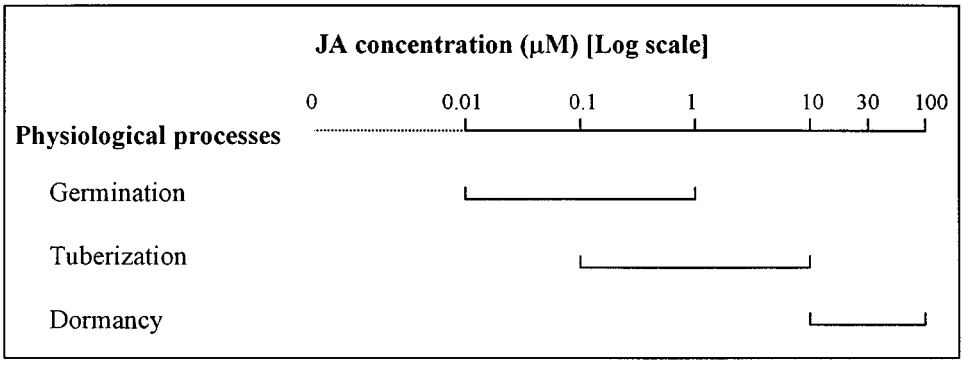
microtubers removed from 30 μM JA reached 80% germination after 10 extra weeks of culture, then 100% after 14 weeks (Figure 2).

In order to evaluate the impact of the exogenous JA treatments on further growth and development, plants from microtubers cultured on JA-free medium and from medium supplemented with 100 μM of JA were transferred to greenhouse conditions. All plants were successfully acclimatized and produced tubers after 8 months of culture in the greenhouse.

In summary, these results show clearly that JA has a dual effect on yam tuber germination. At low concentration, JA promotes germination and at high concentration JA inhibits the germination, possibly by inducing or enhancing the dormancy process. The inhibitory effect of high JA concentration on tuber germination is reversible since germination of the dormant tubers resumes after transfer to JA-free medium (Figure 2). It was reported previously that JA also promotes tuberization in yam and other plant species. The JA concentrations promoting tuberization in *D. batatas* were from 0.1 μM to 100 μM and in potato from 0.1 μM to 10 μM [6, 7]. We therefore examined the JA concentration range affecting the tuberization process in the plant species under investigation. In this respect, node segments of *D. alata* were cultivated in MS medium with various JA concentrations. As shown in Figure 3, the addition of 0.1 or 1 μM of exogenous JA to the culture medium was ineffective. However, addition of 10 μM of exogenous JA, resulted in 100% tuberization after two months of culture. Increasing the JA concentration to 100 μM completely inhibited tubers formation. From examination of the JA concentration ranges for the three physiological processes; germination, tuberization and dormancy (Figure 4), it appears that the yam germination process is promoted by the addition of JA concentrations as small as 0.01 μM. This is the first reported example of JA-induced germination in *Dioscorea* microtubers. The precise physiological implication of JA in the induction of tuberization, dormancy and germination of yam tubers has not been elucidated. Nevertheless other plant growth regulators such as GA_3 have been reported to affect these processes [5, 12]. Both GA_3 (0.1-100 μM) [13, 14] and JA (10-100 μM, our investigation) were found to induce dormancy. However, GA_3 is known to inhibit tuberization [5] and GA_3- dormant tubers require a particular treatment such as incubation in cold temperature in order to germinate. In JA-dormant tubers, germination resumes just after transfer of microtubers to a JA-free medium.

These data demonstrate that exogenous application of JA affects different physiological processes involved in the vegetative propagation of *D. alata* through tubers. The reversible effect of JA on both dormancy and germination has practical significance since this hormonal treatment might be applied to prolong the storage duration of *D. alata* tubers. This potential application is particularly important since difficulty in prolonging the dormancy period constitutes one of the major problems for a profitable management of harvested *D. alata* tubers.

Figure 4. *Illustration of the JA concentration ranges affecting the in vitro germination, tuberization an dormancy of Dioscorea alata microtubers.*



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