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EFFECT OF ABSCISIC ACID, ETHYLENE AND INHIBITORS OF THEIR BIOSYNTHESIS (FLURIDONE AND SALICYLIC ACID) ON SOMATIC EMBRYOS CONVERSION IN TULIPS

WPŁYW KWASU ABSCYSYNOWEGO, ETYLENU ORAZ INHIBITORÓW ICH BIOSYNTEZY (FLURIDONU I KWASU SALICYLOWEGO) NA KONWERSJĘ ZARODKÓW SOMATYCZNYCH TULIPANA

Abstract: The experiments aimed to investigate the conversion of somatic embryos of the tulip 'Apeldoorn' variety in an *in vitro* culture supplemented with some chosen compounds. Tulip somatic embryos in torpedo stage, obtained by indirect somatic embryogenesis were placed for 1 week on media containing growth regulators (5 μM Picloram, 1 μM 6-benzylaminopurine (BAP) – control) and abscisic acid (ABA), abscisic acid + fluridone, Etephon and Etephon + salicylic acid (SA). Then, the embryos were maintained in the dark or under light for 10 weeks.

After time of experiment, the greatest percent of leaf-forming embryos (40 %) and the greatest number of leaves (3.6 leaves) were observed when the embryos were treated simultaneously with Etephon and salicylic acid. Light did not influence the number of newly developed leaves but significantly increased (vs dark) percentage of leaf-forming embryos on control media (from 0 to 24 %) and on Etephon-supplemented medium (from 4 to 24 %). Abscisic acid and abscisic acid + Fluridone supplementation inhibited organogenesis of leaves but only in the cultures maintained under light. Regeneration of leaves was not observed on the control media maintained in the dark and on the abscisic acid + fluridone-supplemented media under light.

Keywords: abscisic acid, ethylene, fluridone, salicylic acid, leaf formation

Reproduction of tulips, commercially important ornamental bulb plants, under natural conditions is a very slow process. Propagation *in vitro* using somatic embryos induction can shorten time required to obtain proper number of bulbs, which is especially valuable in introduction of new cultivars or elite-plant productions. However, this method require further studies because of low conversion of embryos to plants [1, 2], focussed on maturation, germination and conversion of somatic embryos [3]. The conversion of tulip somatic embryos to plants is decisive for efficacy of

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micropropagation. Plants obtained by somatic embryogenesis or shoot cultures [4, 5] are important for production of start material for further propagation. A possibility of cyclic multiplication of tulip auxiliary shoots can increase multiplication index in these plants [6].

The present experiment aimed to examine the effect of growth regulators, abscisic acid (ABA), ethylene (Etephon) and inhibitors of their biosynthesis (fluridone and salicylic acid – SA) on ability of somatic embryos to form leaves during embryo conversion to plants.

Material and methods

The studies were conducted on the tulip of the 'Apledoorn' variety, bulbs of which were maintained at 5 ^{o}C for 12 weeks. Ovaries isolated from bulbs, from flower buds were cut into 1–2 mm pieces and placed in Petri dishes on media containing mineral salts according to Murashige and Skoog [7], 3 % sucrose and growth regulators: Picloram (25 and 50 μM) and BAP (0.25–10 μM). Growth regulators stimulated development of embryogenic callus on explants. When Picloram and BAP concentration was lowered to 5 μM and 1 μM, respectively, the embryogenic tissue formed somatic embryos. Tulip somatic embryos in torpedo stage, 5–10 mm long, were placed on media containing 5 μM Picloram and 1 μM BAP (control) and: 10 μM ABA, 10 μM ABA + 30 μM fluridone, 25 μM Etephon, 25 μM Etephon + 10 μM SA, for 1 week. Then, the embryos were maintained on the medium enriched in 2.5 μM BAP and 0.25 μM NAA in the dark or under light for 10 weeks. Thereafter, the percentage of leaf-forming embryos and the number of leaves were recorded.

The experiment was performed in 5 repetitions, for 5 explants. The results were subjected to the analysis of variance. The means were compared using Duncan test at confidence level $\alpha = 0.05$.

Results and discussion

The embryo germinating under natural conditions first develops cotyledon (with apical bud at the base), root and stolon (an underground shoot). Then the cotyledon dries out and a leaf develops. Tulip seedlings require several years to bloom, until then, the apical bud forms only one leaf [8]. Conversion of somatic embryos consists in simultaneous development of the root and the shoot [9, op. cit. 3].

In present experiment tulip somatic embryogenesis progresses as follows: first, the cotyledon develops, which grows upright, then, it dries out from the top, while a leaf or stolon forms at the base of the embryo. Unfortunately, many embryos do not initiate growth nor develop cotyledon but undergo deformation or die. A majority of tulip somatic embryos do not form leaves or stolons, while those that develop do not have meristem. Similar observations in tulip culture *in vitro* were reported by Podwyszynska and Marasek [6]. The formation of stolons in tulip somatic embryo cultures has been observed sporadically, whereas no root formation was noted. According to Cavallini and Natali [10], problems with normal development and germination of somatic

embryos are observed frequently in monocotyledones. These embryos do not enter dormancy, prematurely germinate, and the plants having developed from them are characterized by low survival rate [11].

The conversion of tulip 'Apeldoorn' somatic embryos was observed on media containing (apart from growth regulators under study) low concentrations of auxins and cytokinins, like Picloram (5 μM), NAA (0.25 μM) and BAP (1 and 2.5 μM). According to Ptak and Bach [12], tulip embryos germinated forming normally developed plants when cultured with 5 μM BAP and 0.5 μM NAA. Picloram (1.4 μM) and BAP (13.3 μM) stimulated the formation of shoots in garlic cultures [13]. Only embryos that have accumulated a sufficient amount of storage reserves can undergo conversion to properly developed plants [3]. The accumulation of storage reserves, decisive for somatic embryo germination yield is stimulated by abscisic acid [14].

Exogenous ABA alone or in combination with fluridone (an inhibitor of its synthesis) inhibited leaf organogenesis of tulip cultures, in present experiment, but only under light (Figs. 1 and 2). According to Stasolla and Yeung [15], proper growth of somatic embryos requires exogenous ABA, concentration of which is species-dependent. ABA promoted the conversion of asparagus [16].

Supplementation of fluridone, an abscisic acid synthesis inhibitor which lowers ABA accumulation in plants [17], had no effect on leaf formation by tulip somatic embryos (Figs. 1 and 2). Probably, its effect was compensated by exogenous ABA, also present in the medium. Gabryszewska [18] noted that fluridone increased the number of leaves in peony cultured *in vitro*, but when it was used in combination with ABA, its action was abolished.

Medium supplementation with Etephon did not affect the percent of leaf-forming embryos or the number of leaves, in tulip 'Apeldoorn' either in the dark or under light (Figs. 1 and 2). Ethylene (released during Etephon breakdown) is considered to be mostly an inhibitor of cell division, but can stimulate some morphogenetic processes. In *Hemerocallis* cultures, ethylene caused transition from young to mature phase, while in

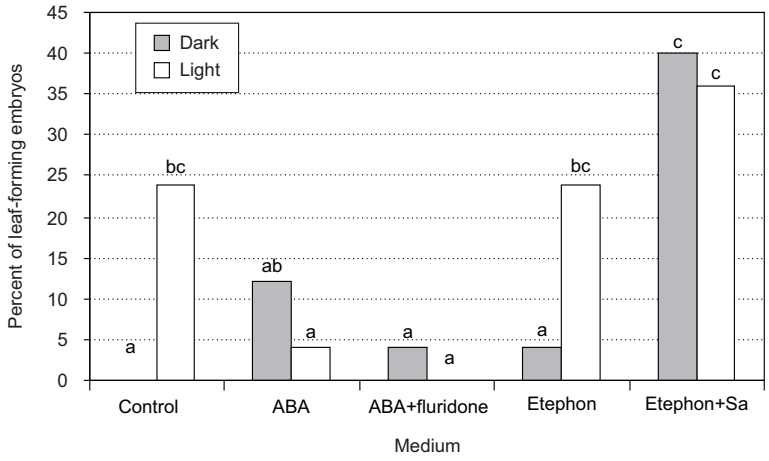

Fig. 1. The effect of growth regulators on leaf formation by somatic embryos of the tulip 'Apeldoorn' variety

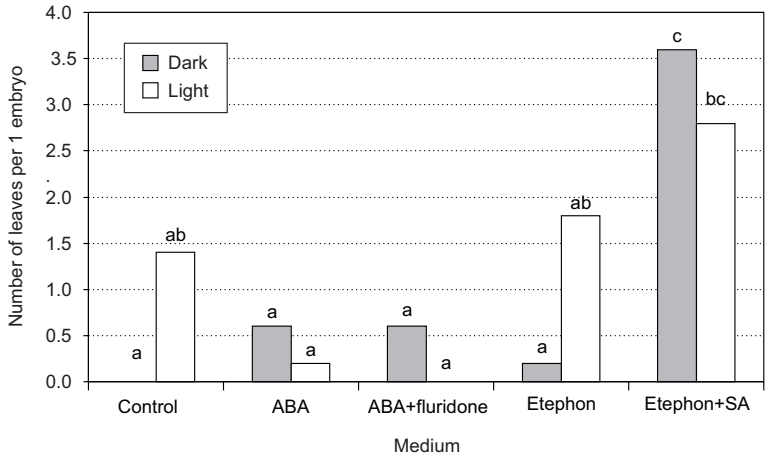

Fig. 2. The effect of growth regulators on the number of leaves formed by somatic embryos of the tulip 'Apeldoorn' variety

roses it stimulated flower bud formation [19]. Ethylene is also a stimulator of seed germination in many plant species [3]. According to Economou [20], ethylene can facilitate shoot formation in some species, however, it should be remembered that its supraoptimal concentrations are ineffective or even inhibitory.

In presented experiment, tulip embryos were exposed simultaneously to Etephon and SA, an inhibitor of ethylene biosynthesis. Medium enrichment in SA resulted in the greatest percentage of leaf-forming embryos (40 %) and the greatest number of leaves (3.6) in the dark. Under light, SA 1.5 times increased the share of leaf-forming explants and elevated the number of leaves, but the differences were not significant vs control medium (Etephon alone) (Figs. 1 and 2). Salicylic acid was shown to have a beneficial effect on cell growth [21]. Light significantly increased the percent of leaf-forming embryos on control medium (from 0 to 24 %) and on Etephon- -supplemented medium (from 4 to 24 %). Leaf regeneration was not observed on control medium in the dark and on medium supplemented with ABA and fluridone under light (Figs. 1 and 2).

Conclusions

1. The greatest share of leaf-forming embryos (40 %) and the greatest number of formed leaves (3.6) were obtained on medium containing Etephon in combination with SA maintained in the dark.

2. ABA and ABA + fluridone supplementation inhibited leaf organogenesis, but only when embryos were cultured under light.

3. Light significantly increased the percentage of leaf-forming embryos under control conditions (from 0 to 24 %) and after Etephon treatment (from 4 to 24 %) in comparison with cultures maintained in the dark.

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Abstrakt: W przeprowadzonym doświadczeniu badano konwersję zarodków somatycznych tulipana ‘Apeldoorn’ pod wpływem wybranych czynników kultury *in vitro*. Zarodki somatyczne tulipana, w stadium torpedy, uzyskane drogą pośredniej embriogenezy somatycznej, wykładano na okres 1 tygodnia, na pożywki zawierające substancje wzrostowe (5 μM Picloram, 1 μM 6-benzyloaminopuryna (BAP) – kontrola) oraz: kwas abscysynowy (ABA), kwas abscysynowy+Fluridon, Etefon i Etefon+kwas salicylowy (SA). Następnie zarodki umieszczano na 10 tygodni w warunkach zaciemnienia lub światła.

Po upływie czasu trwania doświadczenia zaobserwowano, że pod wpływem jednoczesnego działania Etefonu i kwasu salicylowego uzyskano największy udział zarodków formujących liście (40 %) oraz największą liczbę wytworzonych liści (3,6 szt.). Światło nie miało wpływu na liczbę powstałych liści, natomiast istotnie zwiększyło udział tworzących je zarodków na pożywkach kontrolnych (z 0 do 24 %) i po zastosowaniu Etefonu (z 4 do 24 %) w porównaniu z zarodkami utrzymywanymi w ciemności. Dodatek kwasu abscysynowego oraz kwasu abscysynowego i Fluridonu hamował organogenezę liści, ale tylko w obecności światła. Nie obserwowano regeneracji liści na pożywkach kontrolnych w warunkach zaciemnienia oraz pod wpływem kwasu abscysynowego i Fluridonu na świetle.

Słowa kluczowe: kwas abscysynowy, etylen, Fluridon, kwas salicylowy, formowanie liści