Dependence of floral and embryogenic response of *Nicotiana* tissues *in vitro* on phytohormonal impact and photoperiod

D. Raklevičienė,
I. Šaulienė

Institute of Botany,
Žaliųjų ežerų 49,
LT-2021 Vilnius, Lithuania

The effect of the duration of the phytohormonal impact and photoperiod conditions required for the realisation of floral or vegetative organogenesis in thin layer tissues of long-day *Nicotiana alata* L. flower stalks and for the induction of direct somatic embryogenesis in *Nicotiana tabacum* L. leaf tissues was studied. After the phytohormonal impact, there was further cultivation on the medium having no phytohormones. Flower stalk thin layer tissues of *Nicotiana alata* L. were cultivated under a long- (16 h/d light) and short- (8 h/d light) day photoperiod. The phytohormonal impact was made using 1 μM IAA and 1 μM BA which caused the induction of flower and vegetative buds. Irrespective of the ratio of day to night duration, the induction of vegetative buds in *Nicotiana alata* L. flower stalk tissues was determined after 3 days of contact with the auxin- and cytokinin-supplemented medium. Flowers were formed only in long day when the contact of tissues with the phytohormonal medium had been no less than 2 days, but no more than 3–5 buds per explant occurred when the duration of the phytohormonal impact varied from 3 to 30 days. The period required for the induction of direct somatic embryos in tobacco (*Nicotiana tabacum* L.) leaf tissues with exogenously applied 0.5 μM NAA plus 4.4 μM BA reached 8 days, while the maximum frequency was observed only after 15 days. The elimination of the phytohormonal stimulus after determination of the process showed an increase in the number of both organs and embryos.

Key words: phytohormonal impact, photoperiod, thin layer tissues, vegetative and flower buds, somatic embryos

INTRODUCTION

Regeneration processes observed in plant cells and tissues greatly depend on such stimuli as light and phytohormones [1, 2]. The success of organ and embryo neoformation is undoubtedly related to the genetic properties of the object and differentiation peculiarities of the tissues [1, 3]. Cultures of thin layer tissues allow to minimize the influence of tissues differentiated otherwise [4]. Floral features of such explants are directly related to flowering processes occurring in intact plants [3, 5]. Photocontrol of flowering is closely connected with the photoperiod influence on the leaf from which the stimulus is transferred into the apical meristem [6]. Although there has recently been an increase in the knowledge of the photoreceptor signalling the pathway of flowering [7], the relation between light and phytohormone action is not quite clear yet [8]. According to the published data [9], the number of cells capable of carrying out a particular morphogenetic program increases with the extension of the induction period. The interval of the time at which the development is initiated by the phytohormonal impact from the medium and the determined way of differentiation were expected to be fairly constant. The photoregulated expression of the floral and vegetative organogenesis in thin layer tissues of the flower stalk of long-day *Nicotiana alata* L. under different duration of the phytohormonal impact and under a long- or short-day photoperiod has been estimated. Also, the possibilities of somatic embryogenesis in *Nicotiana tabacum* L. leaf tissues under the influence of auxin–cytokinin stimulus have been analysed.

MATERIAL AND METHODS

The experiments were performed with *Nicotiana alata* L. flower stalk thin layer and *Nicotiana tabacum* L. leaf tissue cultures. Intact *Nicotiana alata* L plants

bloomed under a long-day (16 h/d) photoperiod. Thin layer tissues excised from flower stalks of *Nicotiana alata* L. were used. These tissues were cultivated 30 days under 16 h/d long-day (LD) and 8 h/d short-day (SD) light conditions. The number of the newly formed buds in an explant and their biomass were estimated in both vegetative and floral buds. Leaf explants were taken from the 3rd or 4th upper internodes of *Nicotiana tabacum* L. The experiments were carried out with explants of equal size, the parameters of which were: area 7.06 mm^2, average biomass 1.57 ± 0.08 mg. The basal medium of the cultivation contained Murashige and Skoog's (MS) salts [10]. The medium of the thin layer tissues was supplemented with 1 μM indole-3-acetic acid (IAA) and 1 μM 6-benzylaminopurine (BA). Somatic embryogenesis was induced in tobacco leaf explants on MS with 0.5 μM α-naphthaleneacetic acid (NAA) plus 4.4 μM BA [11]. The initiation of vegetative buds, flowers and somatic embryos (SE) was investigated in the experiments when, after the phytohormonal impact, phytohormones were eliminated from the medium, and further growth of tissues (up to 30 days) occurred on the medium having no phytohormones. The duration of the contact of the tissues with the phytohormonal media was 1, 2, 3, 4, 6, 8, 10, 12, and 14 days. Also, the number of structures was determined as explants were grown on the media without or with phytohormones in the course of the whole experiment.

RESULTS AND DISCUSSION

Phytohormones control not only many aspects of plant development, but also their responses to the environment [12, 13]. Realisation of the phytohormonal impact in the organogenesis *de novo* in a long-day *Nicotiana alata* L. flower stalk thin layer tissues cultivated in LD and SD photoperiod was analysed and estimated (Fig. 1). SD effect showed to be more significant on the formation of vegetative buds in comparison with LD. However, flower buds appeared only in LD. The phytohormonal impact on the initial events of morphogenesis was similar under both photoperiods. Irrespective of the ratio of day to night duration and differentiation of the used tissues, organogenesis was not induced without an impact of phytohormones. The appearance of the initial vegetative buds and shoots in thin layer tissues was observed after 3 days of contact with the auxin/cytokinin supplemented medium (13.41 ± ± 1.63 and 11.05 ± 1.18 buds/explant under SD and LD, respectively). Lengthening of the phytohormonal impact revealed differences when the number of buds was obviously influenced by light-to-dark ratio. A smaller yield was in LD as compared with SD.

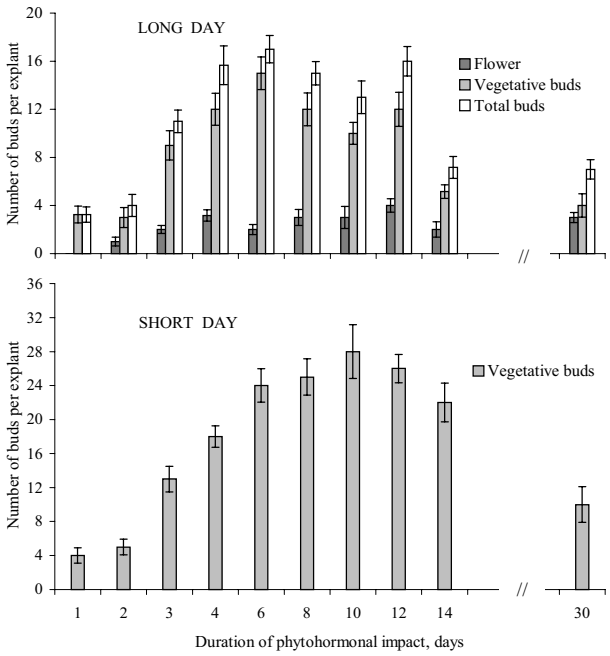

Fig. 1. Effect of the duration of phytohormonal impact and of photoperiod conditions in *Nicotiana alata* L. thin layer tissues on bud formation. Long day – 16 h/d. Short day – 8 h/d

Thus, after 4 days of phytohormonal impact, the differences in bud formation were statistically significant and resulted in 14.22 ± 1.25 and 18.00 ± ± 1.22 buds/explant in LD and SD, respectively. The biggest amount of buds per explant (28.00 ± 3.81) appeared in SD after 10 days of phytohormonal induction. The total number of buds in thin layer explants uninterruptedly cultivated on the media with phytohormones showed no statistical difference under LD and SD conditions (7.59 ± 1.24 and 10.26 ± ± 2.69 buds/expl., respectively). No flowers appeared in SD. A two-day contact with phytohormones was sufficient for the beginning of flower formation in LD. However, no more than 3–5 flowers per explant were formed when the contact of tissues with the phytohormonal medium was extended from 3 to 30 days. After a limited impact, there was no statistically significant increment of flowers. In the same context in other studies it was shown that 4 days were a decisive period in the induction of roots and flowers in thin layer tissues of *Nicotiana tabacum cv.* Samsun [5]. The presented data suggest that after determination neither floral morphogenesis nor the growth intensity of the induced buds can be altered by the phytohormonal impact. However, vegetative buds which formed in SD were significantly smaller in comparison with LD. Thus, the data lead to the assumption that the promotive and repressive pathways control flowering by growth regulators required in appropriate concentrations and at particular times before flowering [2, 6].

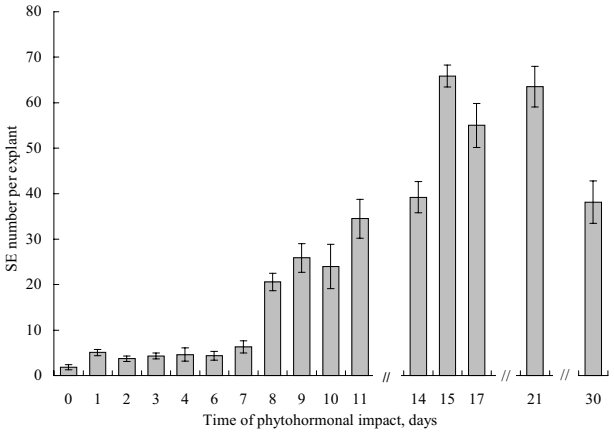

Fig. 2. Effect of the duration of phytohormonal impact on the frequency of direct somatic embryogenesis in *Nicotiana tabacum* L. leaf explants

In studying the morfogenesis, particularly in callus, *Nicotiana* is often attributed to a "model species". However, according to the available information, only three cases of somatic embryogenesis in tobacco tisssues have been described [11]. SE in tobacco callus tissues was induced under intensive lighting conditions, and embryos were obtained from tobacco leaf tissues. In the presented experiments, the duration of the phytohormonal stimulus necessary for initiation of direct somatic embryogenesis was estimated (Fig. 2). No embryos occurred when explants were uninterruptedly grown in the absence of phytohormones. Lengthening of the cultivation period on the phytohormonal medium led to an increased number of SE – from 2–5 SE/expl. after the first seven days to 20.58 ± 1.94 and 25.86 ± ± 3.15 SE/expl. after 8 and 9 days of cultivation, respectively. The highest frequency of SE was observed after 15–21 days of the phytohormonal impact. However, it was significantly lower after 30 days, *i.e*. after uninterrupted phytohormonal influence. Rhizogenesis in the observed SE was slower than the growth and development of shoots under constant phytohormonal conditions. The existence of some critical stages controlled by gene expression and requiring modification of the auxin to cytokinin ratio in SE morphogenesis can testify to the phenomenon of retarded rhizogenesis [14]. Elimination of the phytohormonal stimulus after determination of morphogenetic processes in cultures *in vitro* and their further cultivation on the medium with no phytohormones increased both the number and the growth intensity of embryos. Our experiments suggest that induction of bud and embryo formation with applied phytohormones is determined in time. A very significant, though limited in duration, influence of external phytohormones in the developmental progress under different photoperiodic conditions was demonstrated. However, the interrelation determined by environmental conditions making physiological signals in *in vitro* tissues has not yet been established and provide a basis for the control of morphogenesis.

References

1. Souter M, Lindsey KJ. Exp Bot 2000; 51 (347): 971–83.
2. Levy YY, Dean C. Plant Cell 1998; 10 (12): 1973–89.
3. Altamura M, Angeli S, Capitani F. J Exp Bot 1998; 49 (324): 1139–46.
4. Tran Thanh Van M, Thi Dien N, Chlyah A. Planta 1974; 119: 149–59.
5. Couson A, Tran Thanh Van K, Hanh Trinh T. Physiol Plant 1992; 85: 102–10.
6. Bernier G, Havelange A, Houssa C et al. Plant Cell 1993; 5 (10): 1147–55.
7. Frohnmeyer H. Trends Plant Science 1999; 4 (3): 294–5.
8. Lobello G, Fambrini M, Baraldi R et al. J Exp Bot 2000; 51 (349): 1403–12.
9. Hempel FD, Welch DR, Feldman LJ. Trend Plant Science 2000; 5 (1): 17–21.
10. Murashige T, Skoog F. Physiol Plant 1962; 15: 473–97.
11. Stolarz A, Macewicz J, Lörz H. J Plant Physiol 1991; 137(3): 347–57.
12. Bisseling T, Weigel D. Plant Cell 2001; 13 (2): 221–7.
13. Nato A, Fresneau Ch, Moursalimova N et al. Plant Physiol Biochem 2000; 38 (6), 483–90.
14. Akashi R, Uchiyama T, Sakamoto A et al. J Plant Physiol 1998; 152(1): 84–91.

D. Raklevičienė, I. Šaulienė

FITOHORMONINIO STIMULO IR FOTOPERIODO POVEIKIS ŽIEDŲ IR EMBRIOIDŲ GENEZEI *NICOTIANA* AUDINIUOSE *IN VITRO*

S a n t r a u k a

Tirtas ekzogeninio fitohormoninio stimulo ir fotoperiodo poveikis žiedų ir vegetatyvinių pumpurų genezei *Nicotiana alata* L. žiedkočio plonasluoksniuose audiniuose bei somatinei embriogenezei *Nicotiana tabacum* L. lapo audiniuose. Žiedkočio plonasluoksniai audiniai buvo kultivuojami ilgos ir trumpos dienos fotoperiode. Fitohormonai (1 μM IAR su 1 μM BAP) lėmė žiedų ir vegetatyvinių pumpurų atsiradimą, priklausantį nuo fitohormoninės indukcijos trukmės. Vegetatyvinė organogenezė ilgoje ir trumpoje dienoje nustatyta po 3 parų poveikio fitohormonais. Žiedai formavosi tik ilgos dienos fotoperiode ir jų skaičius neviršijo 3–5 vnt./eksplante. Somatinių embrioidų (SE) indukcijai *Nicotiana tabacum* L. lapo diskuose reikėjo 8 parų kultivavimo terpėse su 0,5 μM NAR ir 4,4 μM BAP. Organų ir SE skaičius didėjo, kai po fitohormoninio stimulo fitohormonai buvo pašalinami iš kultivavimo terpių.