https://doi: 10.17221/38/2017-HORTSCI

Flowering profile and ethylene production of a new carnation subspecies 'Mini-tiara'

Shigeru Satoh[1,*], Yoshihiro Nomura[1], Sayuri Takeuchi[2], Ryusuke Seo[2]

[1]*Faculty of Agriculture, Ryukoku University, Otsu, Shiga, Japan*
[2]*Kagawa Prefectural Agricultural Experiment Station, Ayagawa, Kagawa, Japan*

**Corresponding author: ssatoh@agr.ryukoku.ac.jp*

Abstract

Satoh S., Nomura Y., Takeuchi S., Seo R. (2018): Flowering profile and ethylene production of a new carnation subspecies 'Mini-tiara'. Hort. Sci. (Prague), 45: 187–192.

Ethylene is involved in the senescence of carnation flowers. It is synthesized abundantly after full opening of the flowers and accelerates wilting of petals, which results in withering of the flowers. We investigated the possible involvement of ethylene production in the loss of display value of cut flowers of 'Mini-tiara' carnation, a new subspecies derived from *Dianthus caryophyllus* L. by interspecific cross with wild-type *Dianthus* species (wild pinks). Flowers of 'Mini-tiara' carnation have a unique morphology with pointed-shaped petals, some of which in the middle part of the flowers stand straight and build a dome throughout the display time, and lose their display value when the dome collapses by bending all petals outside (full opening of flowers). Ethylene evolution from 'Mini-tiara' carnation was not detected from flowers with upstanding petals, but occurred several days after the collapse of the dome (after full opening of the flowers), the time they already lost their display value. These findings indicated that ethylene production is not engaged in the loss of display value of 'Mini-tiara' carnation.

Keywords: flower opening and senescence; hybrid carnation; loss of display value; pointed-shaped petals

Ethylene is a primary plant hormone involved in the senescence of cut carnation flowers (Borochov, Woodson 1989; Abeles et al. 1992; Reid, Wu 1992; Satoh 2011). It is synthesized abundantly several days after full opening of the flowers and accelerates wilting of petals. More precisely, in flowers undergoing natural senescence, ethylene is first produced in the gynoecium and induces autocatalytic ethylene production in petals (ten Have, Woltering 1997; Shibuya et al. 2000). Eventually, ethylene produced in the petals accelerates in-rolling of the petals, resulting in wilting of the flowers (Manning 1985; Peiser 1986; Woodson et al. 1992). On the other hand, the effect of ethylene on carnation flower senescence can be diminished by treating flowers with inhibitors of ethylene biosynthesis, prolonging the vase life of the flowers (Satoh et al. 2014). Therefore, the ability of ethylene production in carnation flowers is the primary determinant which regulates the length of vase life (display time) of the flowers.

Recently, a novel carnation subspecies named 'Mini-tiara' was released to the commercial flower market (flower industry) in Japan, and has started to be exported to foreign countries. 'Mini-tiara' carnation was derived from *Dianthus caryophyllus* L. by interspecific cross with wild-type *Dianthus* species at Kagawa Prefectural Agricultural Experiment Station (KPAES), although their parental species were not disclosed presently (KPAES 2016). 'Mini-tiara' carnation has several lines with different petal colors. Flowers of 'Mini-tiara' carnation have pointed-shaped petals, which in the middle part of the flower stand straight during the flow-

Supported by a Grant-in-Aid (No. 16k07604) to SS for Scientific Research from the Japan Society for the Promotion of Science.

https://doi: 10.17221/38/2017-HORTSCI

ers' display time. The flowers are regarded to lose their display value when all the petals bend down, in other words, flowers fully open when all petals are at right angles to the stem.

So far, there have been no reports which describe ethylene production in the flowers and its involvement in the withering (i.e., loss of display value) of 'Mini-tiara' carnation flowers. In the present study, therefore, we investigated the flower opening and senescence profiles and ethylene production during these processes to evaluate the role of ethylene production in the loss of display value during senescence of 'Mini-tiara' carnation.

MATERIAL AND METHODS

Carnation flowers. Two 'Mini-tiara' carnation (*Dianthus* sp.) subspecies, one with pink flowers and another with scarlet ones, named 'Mini-tiara Pink' and 'Mini-tiara Lilac' respectively, were used in the present study. Flowers at the usual commercial stage of flowering, at which the first flower out of six to eight flower buds on a stem was partially open, were harvested with 60-cm long stems in June 2016 at the nursery of Kagawa Prefectural Agricultural Experiment Station in Kagawa Prefecture, Japan. The harvested flowers were transported without supply of water to Ryukoku University in Shiga Prefecture, Japan, two days after harvest. Flowers of 'Light Pink Barbara (LPB)' carnation (*Dianthus caryophyllus* L.), which belong to the spray type of carnation flowers, were obtained from a grower in Miyagi Prefecture, Japan, and transported to the university as described above. After arrival, the flowers were placed in plastic buckets with their cut stem end in tap water until the next day under continuous light from white fluorescent lamps (14 μmol/m^2·s PPFD) at 23°C and 40–70% relative humidity.

Opening and senescence profile. Flowers in a bunch with the stem end in water were left under the conditions described above. Samples of a single flower at each stage of flower opening and senescence (the stages when pointed-shaped petals elongated vertically forming a dome, and susequent collapse of the dome, respectively) were selected and photographed. Photographs of flowers at respective stages were arranged in a row according to the progress of flower opening and senescence.

Assay of ethylene production. Flowers at the respective stages of flower opening and senescence were used for assay of ethylene production. Each stage was defined and named as shown in Fig. 2b.

Particularly, in 'Mini-tiara' carantion, the stage just before flower opening was when two-thirds of the pointed-shaped petals were bent and the remaining one-third still building a dome, whereas in 'LPB' carnation, this stage was when the outermost petals bent at the boundary between the claw and blade (stage 5) (Harada et al. 2010). Florets (single-flowers) with ca. 2 cm stems were detached from whole flower stalks, and placed in 30-ml plastic vials with their cut end in 5-ml ultra pure water (>18 MΩ). Then, the flowers were left under the conditions described above. During this period the water was replenished as necessary and replaced every three or four days. Flowers were photographed daily to record flower opening and subsequent withering of petals.

Ethylene production in the flowers was monitored daily by enclosing individual flowers in 450-ml glass containers (one flower per container) for 2 h at 23°C. Five flowers at each stage of flower opening and senescence were used per carnation cultivar. A 1-ml gas samples were withdrawn with a hypodermic syringe from inside the container through a rubber septum of a sampling port on the container and injected into a gas chromatograph (Model GC-8, Shimadzu, Kyoto, Japan), equipped with an alumina column and a flame ionization detector to determine ethylene content. Fresh weight of each flower was measured before enclosing it in a glass container for the ethylene assay.

RESULTS AND DISCUSSION

Profiles of flower opening and senescence in 'Mini-tiara' carnation

Fig. 1 shows the progress of flower opening and senescence of 'Mini-tiara' carnation flowers. In this figure, the flowers were arranged in a row according to the morphological change of the flowers. The stages progressed similarly in 'Mini-tiara Pink' and 'Mini-tiara Lilac' cultivars. The overlapping petals appeared from the buds at the earliest stage of flower opening [start of flower opening (STO) stage]. When they elongated to about 10 mm, the outermost petals became bent outside at right angles against flower stem. Then inner petals grew gradually to 25–30 mm in length and, during which peri-

https://doi: 10.17221/38/2017-HORTSCI

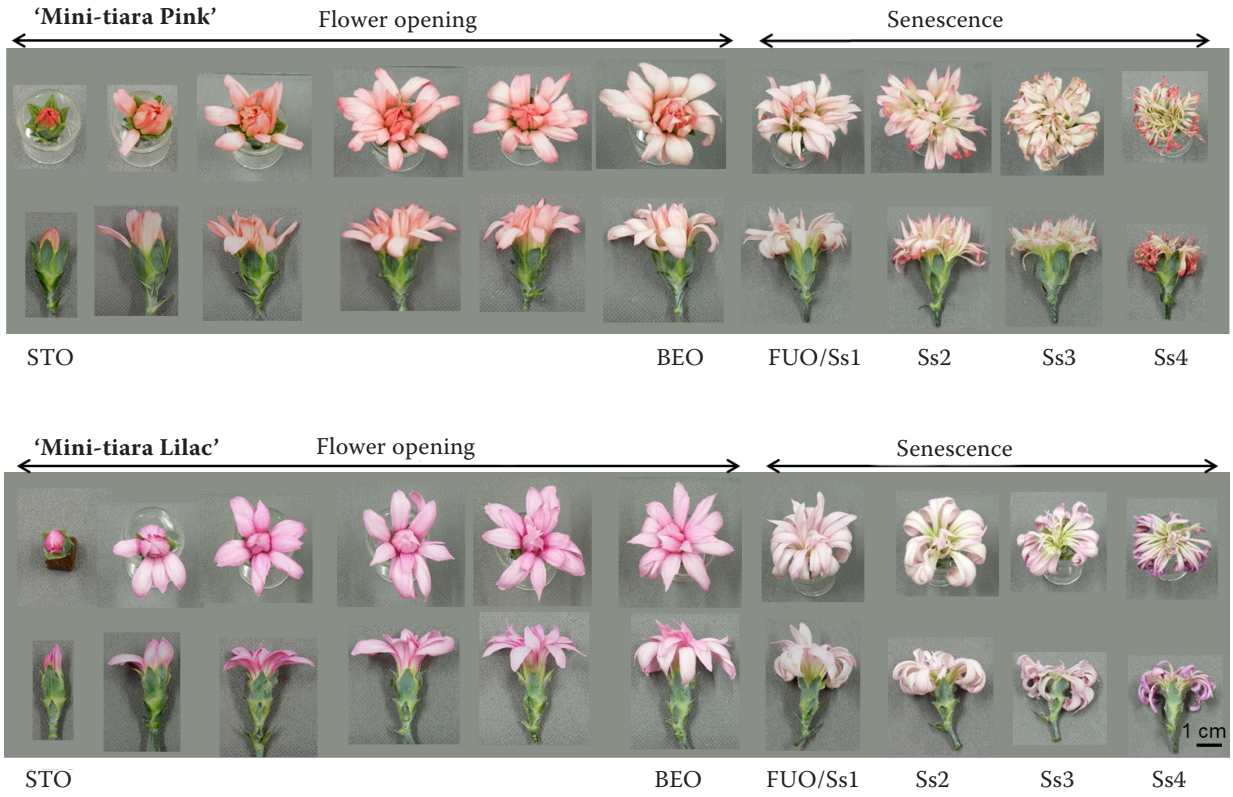

Fig. 1. Profile of flower opening and senescence in the flowers of 'Mini-tiara Lilac' and 'Mini-tiara Pink' carnation flowers
STO – start of flower opening; BEO – before fully-open stage; FUO – fully-open stage; Ss – senescence stage

od, petals bent outside sequentially from outside to inside, until the innermost petals remain standing-upright building a dome [just before the fully-open stage (BEO)]. The last standing petals bent outside and the dome collapsed, the step was the beginning of fully-open stage (FUO). The flowers lost their display values at this stage as discussed below. Furthermore, the flower senescence progressed through 4 distinguished senescence stages (Ss). Ss1 corresponded to the last phase of fully-open flowers (FUO/Ss1) when some petals started to bend down to the calyx tube. At Ss2, most petals were bent down and started to wilt. Petal wilting culminated at Ss3 and some wilted petals were dried up at Ss4.

After a careful inspection we considered that the 'Mini-tiara' carnation flowers have the display value of flowers when they have the unique shape; that is, some of pointed-shaped petals remain standing-upright and building a dome, that is, from STO to before FUO in Fig. 1. We consider that the flowers lose their display value after all of the petals bent down to the calyx tube and the dome collapsed.

Ethylene production in 'Mini-tiara' carnation flowers during flower opening and senescence

We examined the ethylene production in 'Mini-tiara' carnation flowers, in comparison with that in 'LPB' carnation as a reference (Fig. 2a). In this experiment, FUO/Ss1 stage was separated into four stages, i.e., FUO-1, FUO-2, Ss1-1 and Ss1-2, to analyse more precisely ethylene production in flowers at transition from fully-open to senescence stages (Fig. 2b). Five flowers each were used for the respective stages. Ethylene production started at Ss1-2 stage in both 'Mini-tiara Lilac' and 'Mini-tiara Pink' cultivars. Ethylene production rate peaked at Ss2 stage, then declined at Ss3 stage and was not detectable at Ss4 stage with 'Mini-tiara Lilac' cultivar. In 'Mini-tiara Pink' cultivar, however, it peaked at Ss1-2 stage then declined at Ss2 stage, and not detectable at Ss3 and Ss4 stages. The max. ethylene production rates were 0.44 nmol/g FW·h in 'Mini-tiara Lilac' flowers and 0.27 nmol/g FW·h in 'Mini-tiara Pink' ones. On the other hand, in 'LPB' carnation flowers, ethylene production was detected at

https://doi: 10.17221/38/2017-HORTSCI

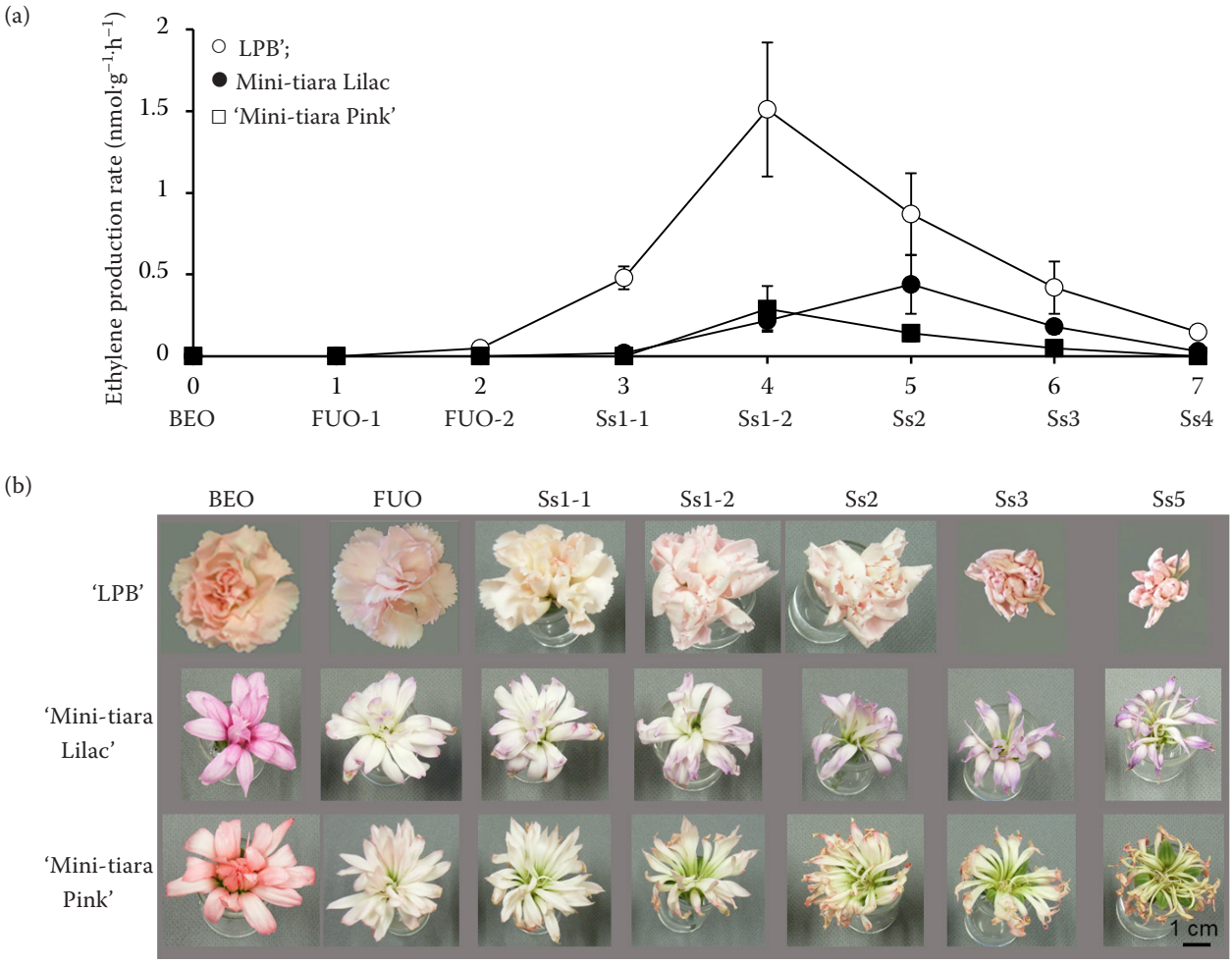

Fig. 2. Ethylene production (a) in 'Mini-tiara Lilac' and 'Mini-tiara Pink' carnation flowers, and appearance of flowers (b) at given stages of flower opening and senescence. As a reference, the results with 'Light Pink Barbara (LPB)' carnation flowers are included. The stages of FUO/Ss1, were further separated into 4 stages, FUO-1, FUO-2, Ss1-1 and Ss1-2 Data are the mean ± SE of five flowers, but 15 flower for 'LPB' flowers; SE is shown by vertical bars when they are larger than the size of symbols

the FUO-2 stage and increased until Ss1-2 stage, attaining 1.51 nmol/g FW·h, then declined thereafter to 0.15 nmol/g FW·h at Ss4 stage. Max. ethylene production rate was much higher in 'LPB' carnation than in 'Mini-tiara' carnation in this experiment.

In Fig. 2a, the ethylene production in 'Mini-tiara Lilac' was slightly later than that in 'Mini-tiara Pink'. Therefore, we again examined the ethylene production rates in 'Mini-tiara Lilac' and 'Mini-tiara Pink' flowers after FUO/Ss1 stage (Fig. 3). In this experiment, ethylene production peaked on day 2 after FUO/Ss1 stage in 'Mini-tiara Pink' flowers, whereas on day 8 in 'Mini-tiara Lilac' ones. The results shown in Fig. 3 were different from those shown in Fig. 2a. However, in both experiments, ethylene production started after the beginning of FUO and earlier in 'Mini-tiara Pink' flowers than in 'Mini-tiara Lilac' ones. In Fig. 3, max. ethylene production rates were 1.21 and 1.91 nmol/g FW·h in 'Mini-tiara Pink' and 'Mini-tiara Lilac' flowers, respectively, and they were much higher than those found in Fig. 2a. The difference in ethylene production rates between the two experiments might be caused by different samples harvested on different days.

It is known that there is a close relationship between the climacteric rise of ethylene production and respiration in senescing carnation flowers (Maxie et al. 1973). However, we did not further investigate the changes of respiration rate in 'Mini-tiara' carnation undergoing flower opening and senescence.

Ethylene plays a causal role for inducing petal in-rolling leading to flower wilting in ordinary car-

https://doi: 10.17221/38/2017-HORTSCI

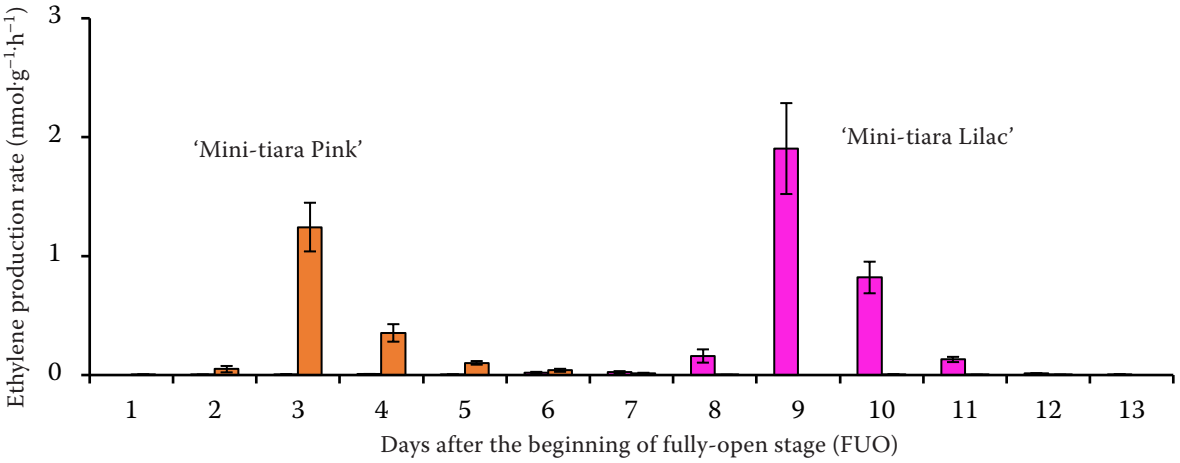

Fig. 3. Changes in ethylene production rates in the flowers of 'Mini-tiara Lilac' and 'Mini-tiara Pink' carnation flowers after the beginning of fully-open stage (FUO)
data are the mean ± SE of five flowers

nation including 'LPB' (Borochov, Woodson 1989; Abeles et al. 1992; Reid, Wu 1992; Satoh 2011). The present study showed that flowers of both 'Mini-tiara Pink' and 'Mini-tiara Lilac' carnation produced ethylene during the senescence (withering) stage (Figs 2a and 3), but ethylene production started far after the flowers had lost their display value at the beginning of FUO, when the dome built with pointed-shaped petals collapsed (Figs 1 and 2b). The profile of ethylene production was similar between 'Mini-tiara' flowers and 'LPB' flowers; ethylene production started at Ss1 or Ss2, distinctly later FUO, in both carnations. However, the morphological difference in flowers between two carnation types causes the difference in time when flowers lose their display values. That is, 'Mini-tiara' carnation loses its display value at the beginning of FOU before the onset of ethylene production, whereas 'LPB' carnation does so at or just after the onset of ethylene production at senescence stage. In other words, ethylene production in 'LPB' carnation flowers started at Ss1 stage, when the flowers still have display value, eventually causing wilting of flowers. This notion indicates that ethylene does not play a substantial role in the loss of display value of 'Mini-tiara' carnation flowers.

CONCLUSION

'Mini-tiara' carnation flowers produce ethylene during senescence, but ethylene production starts after the loss of their display value, indicating little or no involvement of ethylene production in determining the length of the display time.

References

Abeles F.B., Morgan P.W., Saltveit M.E. Jr. (1992): Ethylene in Plant Biology, 2nd Ed. Academic Press, San Diego.

Borochov A., Woodson W.R. (1989): Physiology and biochemistry of flower petal senescence. Horticultural Review, 11: 15–43.

Harada T., Torii Y., Morita S., Masumura T., Satoh S. (2010): Differential expression of genes identified by suppression subtractive hybridisation in petals of opening carnation flowers. Journal of Experimental Botany, 61: 2345–2354.

Kagawa Prefectural Agricultural Experiment Station (2016): Carnation 'Mini-tiara' series. Available at https://www.pref.kagawa.lg.jp/seiryu/kakit-oriatsukai/file/minitiara.pdf (in Japanese)

Manning K. (1985): The ethylene forming enzyme system in carnation flowers (pp. 83–92). In: Roberts J.A., Tucker G.A. (eds): Ethylene and Plant Development. Butterworths, Boston.

Maxie E.C., Farnham D.S., Mitchell F.G., Sommer N.F., Parsons R.A., Snyder R.G., Rae H.L. (1973): Temperature and ethylene effects on cut flowers of carnation (*Dianthus caryophyllus* L.). Journal of the American Society for Horticultural Science, 98: 568–572.

Peiser G. (1986): Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, ACC and ACC-conjugate in cut carnation flowers during senescence. Acta Horticulturae (ISHS), 181: 99–104.

Reid M.S., Wu M.J. (1992): Ethylene and flower senescence. Plant Growth Regulation, 11: 37–43.

https://doi: 10.17221/38/2017-HORTSCI

Satoh S. (2011): Ethylene production and petal wilting during senescence of cut carnation (*Dianthus caryophyllus*) flowers and prolonging their vase life by genetic transformation. Journal of Japanese Society for Horticultural Science, 80: 127–135.

Shibuya K., Yoshioka T., Hashiba T., Satoh S. (2000): Role of the gynoecium in natural senescence of carnation (*Dianthus caryophyllus* L.) flowers. Journal of Experimental Botany, 51: 2067–2073.

ten Have A., Woltering E.J. (1997): Ethylene biosynthetic genes are differentially expressed during carnation (*Dianthus caryophyllus* L.) flower senescence. Plant Molecular Biology, 34: 89–97.

Woodson W.R., Park K.Y., Drory A., Larsen P.B., Wang, H. (1992): Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. Plant Physiology, 99: 526–532.

Received for publication February 23, 2017
Accepted after corrections January 17, 2017