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SHORT COMMUNICATION

Nyctinasty as a tool for reproductive success in *Malachra capitata* L. (Malvaceae)

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Abstract

Two natural populations of *Malachra capitata* (Malvaceae) designated as Group-I and II were studied under daily circadian rhythm as well as artificial night illumination for ascertaining the occurrence of night sleep or nyctinasty in the species and its possible ecological advantage. The Group II population, exposed to 24 hours of illumination evinced higher values for vegetative growth as measured by their leaf size, number of leaves and plant height. However, these same plants expressed reduced or no sleep movement in absence of alternating light and dark phases. On the contrary, the Group I population experiencing natural circadian cycle of 12 hours simultaneous light and dark phase had lower values for the same vegetative growth variables. The plant biomass and flower production values in Group–I plants was higher than that of Group-II. A scatter-plot for flower production and total plant biomass in Group–I had a strong correlation with R value of 0.75. The same correlation for Group–II plants had R-value of 0.27 only. Hence, Group-I plants that experienced night sleep showed a strong and positive correlation between biomass accumulation and flower production. Though the vegetative growth variables were lower for Group I, their higher biomass was contributed mostly by the large number of flowers and the elaborate arrangement of the flowers in the inflorescence. In *M. capitata* the inflorescence has 3-7 heads at each leaf axil. Each head in turn has 2-5 flowers encircled by 3 or 4 conspicuous leafy bracts and bracteoles. The flowers also have a long pedicel and fruits having 5 seeds in the schizocarp. Thus, the reproductive units of the species accumulate a substantial part of the biomass. This is the first report of nyctinasty in *M. capitata* and its assistance in flower production. Hence, it is possible that nyctinasty provides an ecological advantage to the plant species by diverting resources from vegetative activities towards reproductive activities. Therefore, the role of nyctinasty in facilitating biomass accumulation and partitioning of plant resources for reproductive success in plants requires further investigation.

Keywords: Biomass, circadian cycle, nyctinasty, reproductive potential.

Introduction

Nyctinasty is the nocturnal movement of plant parts with the onset of darkness following a circadian cycle in some higher plants. Though nyctinasty was reported from ancient times, it was first studied and illustrated in the plant *Medicago marina* by Darwin (1880). Several other authors have hypothesized nyctinastic movement as a physiological adaptation to abiotic factors such as temperature, moisture, photoperiodism, etc. Darwin suggested that night sleep may impact the thermal balance of the exposed leaf surface on cold nights. Dean and Smith (1978) worked on *Machaerium arboreum* leaves that showed faster drying of vertically placed leaf surfaces on moist nights to avoid the temporary reduction in the rate of photosynthesis caused by wet leaf surface. Some authors proposed that night sleep causes the leaves to be aligned vertically and reduce the shading or safe cover for foraging herbivores (Brown *et al*. 1988, Kotler *et al*. 1991, Taraborelli *et al*. 2003). However, the ecological importance of nyctinasty has not been understood completely.

Nyctinasty has been reported in many plants of *Leguminosae* and *Oxalidaceae* (Zhou *et al.* 2012). The wavelength of the daily incident sunlight, the circadian clock of the species, and the type of phytochrome receiving the incident sunlight are said to control nyctinastic movements (*Satter et al, 1972).* The closing and opening movement of the leaf is mediated through a special motor organ called

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pulvinus, mostly situated at the base of the leaf or leaflet. The pulvinus is composed of two types of tissues; the adaxial flexor tissue and the abaxial extensor tissue. The leaf opening and closing movements are generated by alternating osmotic and plasmolytic conditions of the pulvini motor cells (Uehlein and Kaldenhoff, 2008). The osmotic flux between the pulvinus and surrounding tissue is catalyzed by the potassium ions (K^+). Plants detect red and far red light with the help of its phytochrome. The phytochrome protein undergoes reversible structural changes as Pr and Pfr form depending on red or far red light that is absorbed by it. It has been observed that longer exposure to dark period, leads to low Pfr that allows the leaves to open faster. Masayoshi (2003) reported SLEEPLESS mutation in *Lotus japonicus* (Leguminosae) where the pulvini has been modified into a petiole-like structure resulting in loss of nightsleep. Hence, presence of pulvini is a prerequisite for nyctinasty in plants. Artificial light (Light Emitting Diodes or LEDs) and its spectral quality has been reported to have a dramatic effect on plant anatomy, morphology, nutrient uptake, and pathogen interaction (Massa *et al.* 2008). It is possible that prolonged exposure to artificial night light may also affect the night sleep phenomenon in nyctinastic species.

Relative concentrations of bioactive substances in the motor cells called leaf closing factor (LCF) and leaf opening factor (LOF) affect the nyctinastic movements. In *Phyllanthus urinaria* (Phyllanthaceae) it was observed that leaf-closing factor phyllanthurinolactone changes 20-foldmore at night as compared to the leaf-opening factor phyllurine (Udea and Nakamura 2007). However, it was reported that the time of leaf closing and opening remains somewhat constant. The importance of the pulvinus in leaf movement is also due to the presence of binding sites of leaf movement factors on the surface of the motor cells. Kameyama *et al.* (2000) suggested that the molecular process of bending of the pulvinus may be due to decreased actin tyrosine-phosphorylation in the pulvinus. Since, leaf movement factors are specific to each species the leaf movement factors of *Cassia mimosoides* (Leguminosae) did not recognize the binding sites on the motor cell of *Alibizzia julibrissin* (Sugimoto *et al.* 2001).

A population of *Malachra capitata* (Malvaceae) growing in the fallow land of North 24 Parganas of West Bengal, India was noticed to show upward movement of leaves at sunset. This closing phenomenon of the *M. capitata* was considered unusual as there are no records of nyctinasty in this species. The present study thus attempts to identify the leaf-closing phenomenon in *M. capitata* and explore the probable reasons for such behaviour in the species. Why some plants have nyctinasty and others don't still remain a pertinent question. Hence, the present study also attempts to answer a few underlying questions regarding the benefits of nyctinasty to the plant world. Whether nyctinasty has an impact on plant growth? Whether nyctinasty has an impact on nocturnal protection of the plants? Whether nyctinasty influences the reproductive success of plants? Whether nyctinasty has an advantage over sleepless plants in the whole scheme of survival of the fittest? Does change in the circadian cycle affect the nyctinastic cycle? Thus, this study attempts to answer a few of the above-stated questions by studying two *M. capitata* Groups and exploring their possible nyctinastic behavior and its impact on the growth and flower production of the species.

Methods and materials

A population of *M. capitata* growing naturally in a field of West Bengal, India was identified. One population of the species was called Group-I that experienced a cycle of 12 hours light and 12 hours darkness. Another sub-population under the impact of artificial night light by a 250-watt halogen bulb was considered as Group -II. Group-II plants had 24 hours of exposure to illumination. About 18 plants in Group-I and 20 plants in Group-II were tagged and studied for a period of 6 months. The plants were observed at sunrise and sunset for 15 days to record sleep movement in the species. The tagged plants were measured for recording the plant height and plant girth at 5 cm above ground level. The leaf size, number of leaves and number of flowers per plant were also recorded. The occurrence of disease was recorded for each plant with a *yes* or *no* tag in plants of both Groups. The distance of these plants from the halogen light or any other nocturnal artificial light source was measured. At the end of the study period of six months, the plants were harvested and fresh weight were measured on the same day. The biomass estimation of plants in both Groups was carried out by measuring the dry weight after 25 days.

Results and discussion

M. capitata plats are shrubs growing in moist grounds along fields and fallow lands. They can be easily identified by their large multicostate leaves and yellow flowers in heads with many leafy bracts. The leaves also evince a pulvinus like structure at its point of attachment to the petiole. The Group-I plants that experienced a diurnal light and dark phase of 12 hours exhibited a vertically upward closure of their leaves at sunset and horizontal opening at sunrise (Plate. 1). The leaf closing phenomenon started from 5.00 to 5.30 pm and opening from 4.30 to 5.00 am. The angle of closure of the leaves from the horizontal position ranged from 60^0 to 90^0. The closing and opening of leaves to light exposure or the diurnal circadian cycle observed evinces the process of nyctinasty in the species. Hence, this is the first report of foliar nyctinasty in *M. capitata*. The plants in Group–II under 24 hours illumination showed partial leaf closure at nightfall. The angle of leaf closure ranged from 5^0-15^0 in Group II plants. Thus, continuous light exposure may result in loss of nyctinasty or night sleep phenomenon in the species. The long-term exposure of nyctinastic plants

Plate 1 : Upward closing of leaves at sunset in *M. capitata*

to artificial night light may result in total loss of night sleep in such species.

It was observed that the average height of plants measured in Gr-II was greater (89±19 cm) as compared to plant height in Group-I plants (70±7 cm). The average girth at 5 cm above ground was 4±1 cm and 5±4 cm, respectively in Group-I and Group-II plants. The average number of leaves was 19±4 leaves and 24±8 leaves per plant in Group-I and II, respectively. The average leaf sizes were 64±3 cm^2 and 75±15 cm^2 in Group I and II, respectively. Hence, it can be concluded that Group-II plants that were exposed to night illumination exhibited an increase in the plant height, collar diameter, size and number of leaves.

The increase in growth variables such as leaf size and plant height are likely due to increased photosynthetic activity of Group- II plants exposed to longer period of illumination. Post-harvest the average fresh weight of Group II samples was 57 gms/ plant, and that of Group- I was 42 gms/plant. Likewise, the average dry weight of Group- II samples was 20 gms/plant, and that of Group-I was 26 gms/plant (Figure 1a). The dry weight of the samples was

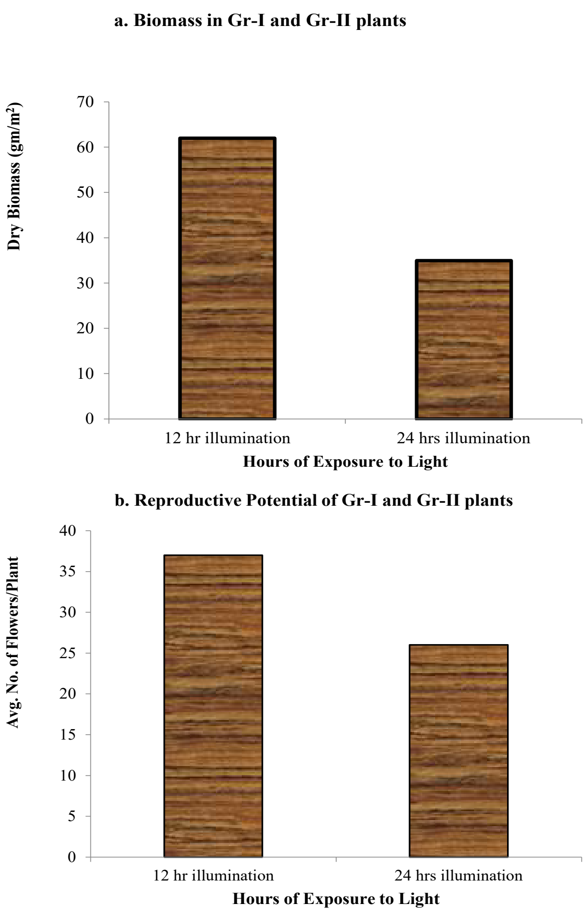

Figure 1: (a) The biomass represented as dry weight (gms/m^2) and (b) Reproductive potential represented as number of flowers/plant for *M. capitata* plants in G-I (12 hrs illumination) and G-II (24hrs illumination).

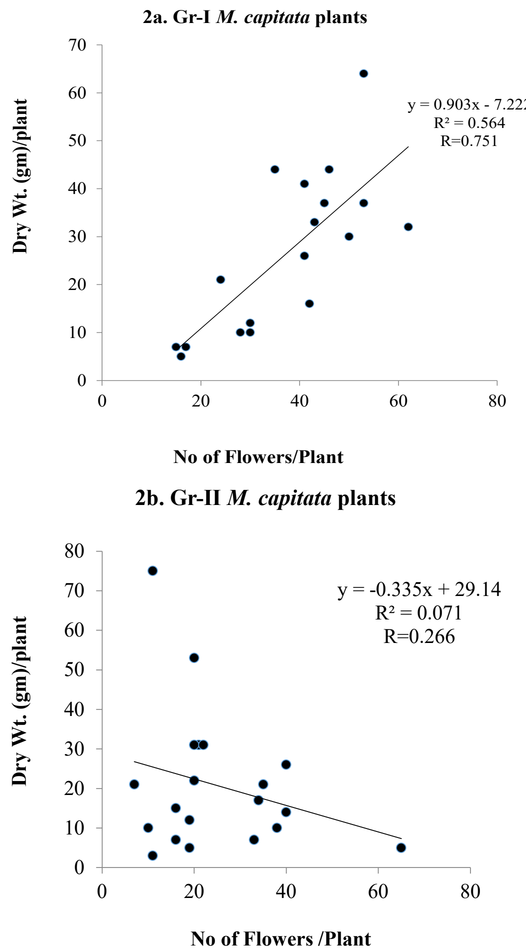

Figure 2: The correlation between plant biomass and reproductive potential of *M. capitata* in Group-I plants (2a) and Group-II plants (2b)

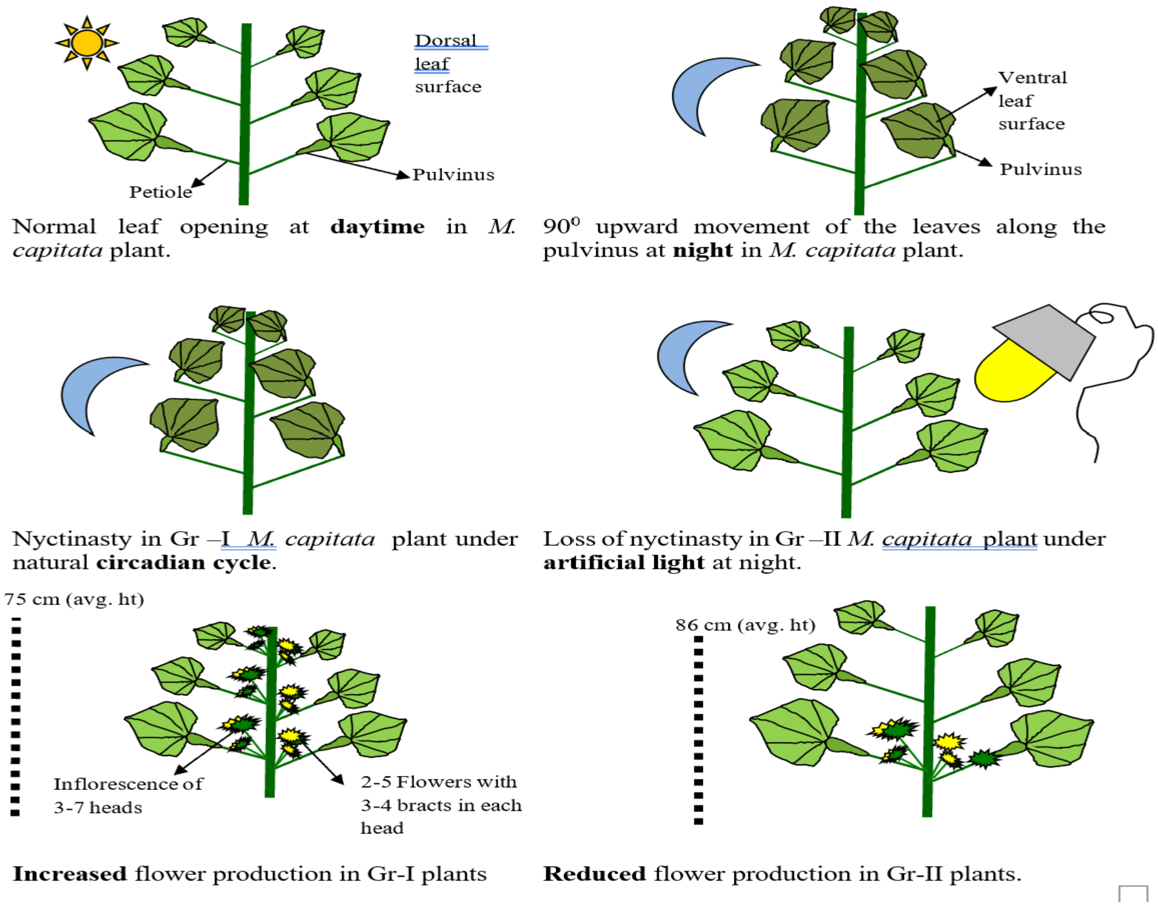

Figure 3: Nyctinasty and its impact on reproductive potential of *M. capitata* (Malvaceae)

calculated to estimate the actual biomass of the samples. The biomass of Group I was calculated as 62gm/m^2 and Group II as 35gm/m^2. Group-II samples having higher growth variables exhibited lower biomass. Whereas, Group I samples having lower growth values exhibited higher biomass. It needs to be mentioned here that the vegetative (leaf and stem) as well as reproductive (inflorescence) structures of the harvested plant samples were measured together for biomass estimation. The average number of flowers per plant was recorded as 37 flowers/plant in Group I and 26 flowers/plant in G-II samples (Figure 1b).Thus, the above results show that the biomass and number of flowers produced by Group-I plants was higher than that of Group-II plants.

The scatter-plot for number of flowers in relation to their biomass in Group-I plant samples exhibited a strong correlation with R value of 0.75. There exists a strong relationship between the reproductive potential and biomass accumulated by the plants in Group-I plants under normal circadian cycle (Figure 2a). Whereas, the correlation between biomass and flower production in Group-II plants expressed an R value of 0.27, only (Figure 2b).

This suggests that the co-relation is weak between the biomass accumulated and the reproductive potential of Group II-plants that did not experience the natural circadian cycle. The higher biomass accumulation in Group I plants under natural nyctinastic process is in tune with earlier study by Zhou *et al.* (2012) stating that nyctinasty helps in plant growth. However, in the present study the higher biomass accumulation in Group –I plants was invested in the reproductive organs as compared to the vegetative organs.

This higher biomass of the Group I plants can be explained by its higher number of flowers produced per plant. Interestingly, in *M. capitata* the flower is an inflorescence with 3-7 heads at each leaf axil. Each head in turn has 2-5 flowers encircled by 3 or 4 conspicuous leafy bracts and bracteoles. Also, each flower has about five seeds in a schizocarp. Hence, the higher biomass of Group I plants is due to its higher number of flowers or reproductive units that not only have flowers and seeds but also leafy bracts and elongated flower pedicels (Figure 3). Hence, it is likely that the sleep movement in the plants allows higher accumulation/assimilation of bioresources at night to contribute towards the reproductive activity of the plant by facilitating higher flower production and seed set.

It may be stated that night sleep in *M. capitata* ensures resource utilization for reproductive purposes. The overall impact of artificial light in Group–II resulting in loss of nyctinasty and reduced flowering potential gives a better understanding of the role of nyctinasty in maintaining the reproductive potential in *M. capitata* (Figure 3). Another, hypothesis put forward by Minorsky (2018) states that nyctinasty could be a plant behavior to discourage insect hervibory. In the present study Gr-I shows 26% higher occurrence of diseases than Gr-II. However, this result is based on the incidence of disease and not on its magnitude.

Conclusion

Nyctinasty has a narrow taxonomic and ecological niche. It is likely that nyctinasty has evolved in some species as a means for improving the reproductive potential of the plants by conserving resources/energy through hibernation at night. In *M. capitata,* plants that experience nyctinasty show higher biomass accumulation and higher flower production. Whereas, *M. capitata* population under a disturbed circadian cycle shows reduced biomass allocation for reproductive activities as well as reduced nyctinastic behaviour. The loss of nyctinastic activity may be temporary or permanent and requires further studies. Hence, the present study opens areas of research on the impact of nyctinasty not only on plant growth but also on their reproductive success. It is clear from the above study that *M. capitata* has evolved with a night sleep that can be disturbed by continuous exposure to bright artificial light. Artificial night light could be a problem to all plants that have evolved with nyctinasty. Since the sleep movement of plants are not related to local weather and follow their circadian cycle it is possible that leaf closing factors (LCF) may be used for developing effective sleeping drugs for humans through in-silico drug designing. The findings of this study could strengthen the importance of night sleep on the fecundity of a species for its overall performance and survival in an ecosystem. However, climate change and over-illumination of urban areas at night may negatively affect this unique evolutionary adaptation of some plants.

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