International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319 – 6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 9; September 2017; Page No. 5995-5998
DOI: http://dx.doi.org/10.24327/ijcar.2017.5998.0849

Research Article

ROLE OF MAJOR FLOWERING TIME GENES IN TOLERANCE OF COPPER STRESS IN MUTANTS OF *Arabidopsis thaliana*

Ankita Rai*

Amity Institute of Biotechnology, Amity University, Noida, India

A R T I C L E I N F O

Article History:

Received 4th June, 2017
Received in revised form 3rd July, 2017 Accepted 24th August, 2017
Published online 28th September, 2017

Key words:

Photoperiodic flowering, Phytoremediation, Accumulation, Physiology

A B S T R A C T

Heavy metal such as copper acts as stress to plants if present in excess concentration in soil and thereby badly affects the plant physiology. In the present work, efforts has been made to study the role of major flowering time genes in tolerance of copper stress in mutants of *Arabidopsis thaliana* by sowing their seeds in soil and then exposing them to varying concentrations (0µM, 25µM, and 150µM) of copper sulfate solution after an adequate period of growth and development. Recent work has started to uncover molecular mechanisms that govern the process of photoperiodic flowering in *Arabidopsis thaliana.* The results obtained were compared to that of wild type (*col-0*). It facilitates the assessment of essential morphological features affected by copper stress such as bud development, flowering time, various symptoms associated with excess copper uptake and chlorophyll density in a single experiment. This study was quite useful in determining the potential of these mutants for use in phytoremediation. This can lead to decrease in the accumulation of heavy metals in the soil thereby enhancing the fertility of the soil and significant increase in the agricultural productivity.

INTRODUCTION

Arabidopsis thaliana is a small flowering plant that belongs to the Brassicaceae family and it is widely used as a model system of preference for research in the field of plant sciences. Considerable advances in comprehending plant growth and development have been made by emphasizing on the genetic and molecular level of this simple angiosperm. In the present work, efforts has been made to examine the role of major flowering time genes in tolerance of copper metal stress in different mutants of *Arabidopsis thaliana* and whether these mutants can be utilized for further use in phytoremediation processes. Genes such as Gigantea (*GI*), Constans (*CO*) and Phytochrome-b (*PHY B*) play a vital role in various important physiological processes like flowering time regulation, hypocotyl elongation, control of circadian rhythm, etc (Priyanka Mishra *et al*., 2015). Thus, the mutant types of these genes such as *gi-100*, *co-10* and *phyb-9* are targeted respectively for this study to get a clear understanding of the tolerating capacity of such mutants in presence of different concentrations of copper. Recent work has started to uncover molecular mechanisms that govern the process of photoperiodic flowering in *Arabidopsis thaliana.* The capability of a plant to induce flowering by assessing the alterations in day-length is referred to as photoperiodic flowering (Thomas *et al*., 1997). Two distinct models have been proposed to elucidate the mechanisms governing photoperiodism: the external coincidence model and the internal coincidence model (Mariko Sawa *et al*., 2008). The external coincidence model suggests that light acts as an external stimulus that corresponds to a light-sensitive rhythm at certain times of day. Flowering is induced in long day plants when they are exposed to light at the crucial point of the rhythm. At the molecular level, the basic mechanism that promote photoperiodic flowering is the induction of *FLOWERING LOCUS T (FT)* gene expression by CONSTANS (CO) protein, which can be best elucidated by the external coincidence model. The *CONSTANS (CO) gene* expression is induced by the circadian clock, and stability and functionality of CO protein are induced by light (Suarez-Lopez *et al*., 2001). In the presence of light, FKF1-GI complex formation triggers the activation of *CONSTANS* gene expression that further stimulates *FT* transcription under long day conditions due to which flowering is induced in *Arabidopsis thaliana.* There is an external coincidence of FKF1 (Flavin Binding Kelch Repeat F-Box 1) protein expression and light. Since FKF1 acts as a photosensitive receptor that senses blue light and mediates the interaction with GI, FKF1-GI complex formation is elicited by blue light. A huge amount of the complex is produced under long day conditions (16 h of light and 8 h of darkness) as compared to short day conditions (8 h of light and 16 h of darkness) due to coincidence of FKF1 expression and light (Aidyn Mouradov *et al*., 2002). The internal coincidence model suggests the coincidence of FKF1 and GIGANTEA (GI) protein expression (Sawa M *et al*., 2007). The two rhythms are brought into the same phase to induce flowering in case of

Corresponding author:* **Ankita Rai
Amity Institute of Biotechnology, Amity University, Noida, India

inductive photoperiods. These models make sure that large quantity of FKF1-GI complex is formed in long day conditions. In SD conditions, GIGANTEA protein expression reaches maximum around 7 h after inception of light, which is about 3 h prior to peak FKF1 protein expression. Therefore, GI and FKF1 expression patterns exhibit less overlap under short day conditions (Samach *et al*., 2000). Also, FKF1 expression takes place mostly in the dark phase leading less amount of formation of the FKF1-GI complex in short day.

During the course of the study, different phenotypic changes have been observed in *gi-100*, *co-10* and *phyb-9* mutants at varying concentrations of copper sulfate solution as compared to that of control. *Arabidopsis thaliana* ecotype Columbia *(col-0)* was used as wild type in this study. Since copper (Cu) is an essential element in plants that is involved in numerous physiological processes like electron transport chain, mitochondrial respiration, oxidative stress responses and hormone signaling, adverse effects were not observed at the initial stage. This may be because adequate amount of copper is required by plants for its proper growth and development (Aula Dei (2005)). In one of the review it has been stated that the average amount of Cu in plant tissue is 10 $\mu g.g^{-1}$ dry weight (Baker DE *et al*.,1995). Thus it is essential that the copper ions must be transported throughout the plant, distributed and compartmentalized within different tissues and its concentration should be cautiously optimized within different cells and organelles. To accomplish this purpose, plants exhibit certain homeostatic mechanisms to retain the accurate concentrations of essential metal ions. But after certain interval of time, certain morphological changes have been observed in plants that were exposed to 25µM and 150µM concentration of copper sulfate solution. They began to develop certain symptoms associated with excess copper uptake like chlorosis caused due to interference of excess copper with metabolism of iron, necrosis, reduction in the number of leaves, delay in flowering time and reduction in chlorophyll density. Copper can become extremely toxic at higher concentrations due to its redox properties. The concentration of copper increases in soil due to the excessive release of heavy metals into the environment through smelting, mining, manufacturing, and agriculture industries (Aula Dei (2005)). These sectors release their effluents directly into the environment without prior treatment resulting in a significant increase in the level of copper in soils. Most of the crop plants face the problem of copper toxicity due to the excessive use of Bordeaux mixture (copper sulfate and lime) as fungicide (Larry P *et al*., 1976). Reuther *et al*. (1953) found the occurrence of chlorosis in citrus seedlings when the amount of total copper of a sandy soil of pH 5.0 exceeded 150 ppm. At the cellular level, it can cause oxidative stress in plants by enhancing the formation of oxygen free radicals resulting from the reaction between superoxide and hydrogen peroxide due to which antioxidant response increases (Aula Dei (2005)). Since copper is both an essential as well as toxic element, diverse techniques have developed in plants to properly maintain its homeostasis as a function *Arabidopsis thaliana* of the ecological copper level (Aula Dei (2005)). The present study facilitates the assessment of essential parameters affected by copper stress such as lateral root development, shoot biomass, bud development, flowering time, various symptoms associated with excess copper uptake and chlorophyll density in a single experiment.

MATERIALS AND METHODOLOGY

Materials Required

Arabidopsis thaliana ecotype Columbia (*col-0*) was used as wild type and Gigantea (*gi-100), Constans (co-10), Phytochrome b (phyb-9)* mutant types were used in this study. 24 small sized pots and 2-trays were taken. Composite mixture of soil was taken for sowing the seeds of different mutants of *Arabidopsis thaliana.* For this, 100 ml of 0.5 M stock solution of Copper Sulfate Pentahydrate was prepared using distilled water after which the working solutions of two different concentrations such as 25µM and 150µM were prepared in 1000 ml of distilled water. Other materials that were required during the procedure are plastic sheets for wrapping the pots, tooth picks, scissor and 10 ml pipette.

METHODOLOGY

The experiment was done in duplicates. For this, 24 small sized pots and 2 trays were taken. 6 pots were taken for each mutant type out of which 2 pots were assigned for each different concentrations of copper. The pots were then wrapped with plastic sheets so as to avoid leakage of water. Then the composite mixture of soil was filled into each pot. Then distilled water was added into the soil dropwise. Seeds of different mutants were taken in very small amount on a piece of paper. Then 5 seeds of *columbia* wild type (*col-0*), *phytochrome-b* (*phy-b9*), *constans (co-10*) and 10 seeds of *gigantea (gi-100)* were picked and sown into the soil with the help of a toothpick. The trays were then covered with polythene bags and the open end was sealed with tape. The trays were then placed inside the cold room at 4˚C for 2 days. After 2 days, the trays were taken out and transferred into Long Day room (16 hours light, 8 hours dark) at 21˚C for around 4 days depending upon the germination process of seed. Then 0.5 M, 100 ml stock solution of Copper Sulfate Pentahydrate salt was prepared after which the working solutions of two different concentrations such as 25µM and 150µM was prepared in1000 ml of distilled water (Paula Duque (ed.), 2016). On 5th day, the plastic bags were removed and around 10 ml of distilled water was added into the soil with the help of pipette. Around 10 ml of water was added consecutively for 3 more days into each pot for their proper growth and development. Likewise on 9th day, around 10 ml heavy metal solution was added into each pot except those labeled as 0µM concentration (CONTROL). On 10th day, again 10 ml of water was added into each pot. (The addition of water and heavy metal solution was done alternately). Then the pots were regularly observed for any significant changes in them at different concentrations of copper.

RESULT AND DISCUSSION

Figure 1 shows the comparison between *col-0* wild type and *gi-100* mutant based on their phenotype

- ***col-0*:** The plants of Wild type (*col-0*) were more healthier as compared to the plants of *gi-100* mutant type as shown in figure 1.1 and 2.1.
- ***gi-100*:** Significant changes were observed in the phenotype of this mutant. The plants appeared more green in color at 0µM indicating high chlorophyll density as compared to that of plants at 25µM (light green in color) and 150µM (yellowish green in color) concentration as shown in figure 1.2 and 2.2. This

illustrates that *gi-100* has a positive role in coping up with copper stress.

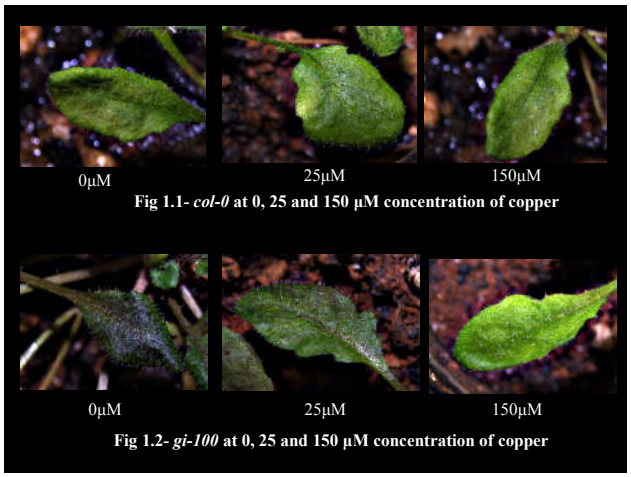

Fig 1 Microscopic view of leaves of *col-0* and *gi-100* mutants of *Arabidopsis thaliana* at varying concentrations of copper. Photos were taken 38 d after exposure of plants to varying concentrations of copper sulfate solution

Fig 2 Comparison between *col-0* wild type and *gi-100* mutant of *Arabidopsis thaliana* based on their phenotype. Photos were taken 30 d after exposure of plants to varying concentrations of copper sulfate solution

Figure 3 shows the comparison between *col-0* wild type and *co-10* mutant based on their phenotype

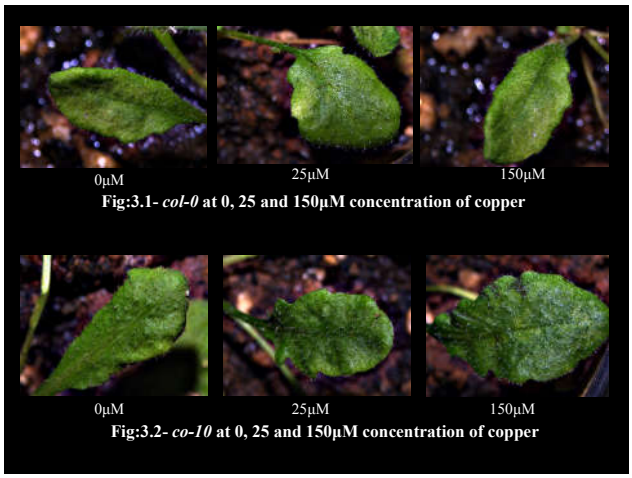

Fig 3 Microscopic view of leaves of *col-0* wild type and *co-10* mutant of *Arabidopsis thaliana* at varying concentrations of copper. Photos were taken 38 d after exposure of plants to varying concentrations of copper sulfate solution.

- ***col-0*:** The plants of wild type mutant were green and healthy as shown in figure-3.1 and 4.1.
- ***co-10:*** No significant changes were observed in this mutant. All the leaves were quite similar to each other in terms of appearance (phenotype) as shown in figure 3.2 and 4.2. This illustrates that *co-10* has a negative role in coping up with copper stress.

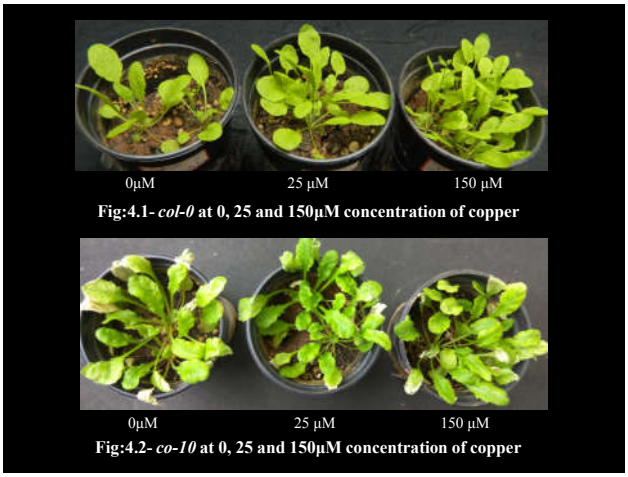

Fig 4 Comparison between *col-0* wild type and *co-10* mutant of *Arabidopsis thaliana* based on their phenotype. Photos were taken 30 d after exposure of plants to varying concentrations of copper sulfate solution.

Figure 5 shows the comparison between col-0 wild type and phyb-9 mutant based on their phenotype

- ***col-0:*** The plants of wild type mutant were healthy and green at all the concentrations of copper. They were quite similar to each other in terms of appearance (phenotype) as shown in figure 5.
- ***phyb*-9:** At 25µM concentration of Copper, the plants were much more healthier as compared to the plants at 150µM concentration as shown in figure 6. Since copper is an essential element, so it is quite possible that *phyb-9* mutant may be able to tolerate or metabolize copper upto 25µM concentration and then at 150µM, it may not be able to tolerate it thereby resulting in symptoms associated with excess copper uptake i.e. chlorosis and necrosis.

Fig 5 *col-0* wild type at 0µM, 25µM and 150µM concentration of copper. Photos were taken 38 d after exposure of plants to varying concentrations of copper sulfate solution

Fig 6 *phyb-9* mutant type at 0µM, 25µM, 150µM concentration of copper. Photos were taken 38 d after exposure of plants to varying concentrations of copper sulfate solution

CONCLUSION

In the present report, the role of major flowering time genes in tolerance of copper stress in mutants of *Arabidopsis thaliana* has been observed based on the phenotypic changes. Also, the various symptoms associated with excess copper uptake such as chlorosis and necrosis has been closely observed. A comprehensive understanding of metal transport in plants and further investigation is still need to be required to determine whether these mutant plants will be able to accumulate specific metals, for use in phytoremediation. This can be done by performing chlorophyll estimation test through Raman's Spectroscopy. This can lead to decrease in the accumulation of heavy metals in the soil thereby enhancing the fertility of the soil and significant increase in the agricultural productivity worldwide.

References

1. Aidyn Mouradov, Frédéric Cremer, and George Coupland, (2002). Control of Flowering Time. The Plant Cell, American Society of Plant Biologists, 14 (Suppl): s111–s130.
2. Aula Dei (2005).Copper in plants. *Brazilian Journal of Plant Physiol*. vol.17 no.1 Londrina, ISSN 1677-9452.
3. Baker DE, Senef JP (1995) Copper. In: Alloway BJ (ed), Heavy metals in soils, Blackie Academic and Professional, London, pp.179-205.
4. Larry P. Gough, Hansford T. Shacklette, and Arthur A. Case (1979). Element Concentrations Toxic to Plants, Animals, And Man, Geological Survey Bulletin, United States government printing office, Washington; pp-22-24.
5. Mariko Sawa, Steve A Kay, and Takato Imaizumi, (2008). Photoperiodic flowering occurs under internal and external coincidence. Plant Signaling and Behaviour, Apr; 3(4): 269-271.
6. Paula Duque (ed.),(2016). Environmental Responses in Plants: Methods and Protocols, Methods in Molecular Biology. Assessing Tolerance to Heavy-Metal Stress in *Arabidopsis thaliana* Seedlings" vol. 1398, Springer Science+Business Media New York, Chapter-16, Humana Press, 197-208.
7. Priyanka Mishra and Kishore C. Panigrahi, (2015). GIGANTEA – an emerging story. Front Plant Sci.; 6: 8.Published online 2015 Jan 26.
8. Reuther, Walter, and Smith, E E, (1953). Effects of high copper content of sandy soil on growth of citrus seedlings: Soil Sci., v. 75, p. 219-224.
9. Samach, A., and Coupland, G. (2000). Time measurement and the control of flowering in plants, Bioessays 38-47.
10. Sawa M, Nusinow DA, Kay SA, Imaizumi T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis, *Science*.; 318:261-26.
11. Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F., and Coupland, G. (2001). CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*, Nature 410 1116–1120.
12. Thomas, B., and Vince-Prue, B. (1997). Photoperiodism in Plants, 2nd ed. (San Diego, CA: Academic Press).

How to cite this article:

Ankita Rai (2017) 'Role of Major Flowering Time Genes in Tolerance of Copper Stress in Mutants of Arabidopsis thaliana ', *International Journal of Current Advanced Research,* 06(09), pp. 5995-5998.
DOI: http://dx.doi.org/10.24327/ijcar.2017.5998.0849

****