COVID-19 patients. Clinical studies for testing this hypothesis are the next step.

1. Napoli, P. E. and Nioi, M., *J. Clin. Med*., 2020, **9**(4); doi:10.3390/jcm9041138.
2. Jha, P. *et al*., *Infect. Genet. Evol*., 2012, **12**(1), 142–148; doi:10.1016/j.meegid.2011.11.001.
3. Kidd, J. M., Newman, T. L., Tuzun, E., Kaul, R. and Eichler, E. E., *PLoS Genet*., 2007, **3**(4), e63; doi:10.1371/journal.pgen.0030063.
4. Milewska, A. *et al*., *Sci. Rep*., 2018, **8**(1), 5960; doi:10.1038/s41598-018-24448-2.
5. Wang, K. *et al*., *PLoS ONE*, 2013, **8**(5), e64809; doi:10.1371/journal.pone.0064809.
6. Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. and Soubrier, F., *J. Clin. Invest*., 1990, **86**(4), 1343–1346; doi:10.1172/JCI114844.
7. Adamzik, M. *et al*., *Eur. Respir. J*., 2007, **29**(3), 482–488; doi:10.1183/09031936.00046106.
8. Li, X., Sun, X., Jin, L. and Xue, F., *Eur. J. Hum. Genet*., 2011, **19**(9), 1002–1008; doi:10.1038/ejhg.2011.66.
9. Hatami, N. *et al*., *Endocrine*, 2020, **68**(3), 479–484; doi:10.1007/s12020-020-02381-7.
10. Revelas, M. *et al*., *Mech. Ageing Dev*., 2018, **175**, 24–34; doi:10.1016/j.mad.2018.06.002.
11. Clarke, N. E. and Turner, A. J., *Int. J. Hypertens*., 2012, **2012**, 307315; doi:10.1155/2012/307315.

ACKNOWLEDGEMENTS. We thank Prof. Samir K. Brahmachari for critical comments and Rintu Kutum for the figure. This is CDRI communication no. 10099.

Received 24 July 2020; accepted 29 July 2020

SAMAN HABIB[1,*]
MITALI MUKERJI[2,*]

[1]*Division of Molecular and Structural Biology,*
Sitapur Road,
CSIR-Central Drug Research Institute,
Lucknow 226 031, India
[2]*Genomics and Molecular Medicine,*
CSIR-Institute of Genomics and Integrative Biology,
Mathura Road,
New Delhi 110 025, India
**For correspondence.*
e-mail: saman_habib@cdri.res.in; mitali@igib.res.in

Rehydration induces early and rapid bud break in drought stressed mulberry plants

Bud break is a natural phenomenon in trees to re-activate growth in response to favourable changes in the environment. Trees enter the growth arrest stage called dormancy, as an adaptive strategy to survive the unfavourable environmental conditions. In temperate trees, bud break is triggered after winter dormancy[1], whereas in tropical trees, these phenological events mostly depend on water availability[2,3]. In plants, under unfavourable conditions like desiccation stress, one of the earliest responses is the accumulation of abscisic acid (ABA), an endogenous growth hormone[4]. ABA is a long-distance signalling hormone which is generally transported from roots to shoots resulting in growth arrest[5]. In addition to ABA, ethylene also has a key role in triggering the induction of bud dormancy and repressing bud activity[6], thereby delaying bud break, whereas gibberellin (GA) induces dormancy release[7]. Therefore, it is likely that the buds from the plants grown under ideal conditions would exhibit early and rapid bud break compared to those from the drought-stressed plants. We tested this hypothesis in mulberry (*Morus alba* L.), a commercially important perennial system, where periodic pruning is required to regenerate vegetative growth[8–10]. In India, most of the mulberry cultivation falls under arid or semi-arid conditions, where the plants are routinely exposed to intermittent drought that adversely affects foliage production[11,12]. To examine the effect of drought stress on bud break, we created two different levels of soil water status (100% and 40% field capacities, FC) in potted mulberry plants by gravimetric approach[13], thereby simulating drought stress. The pots were maintained in open field and at ambient conditions, while being protected from rain with the help of rain out shelters. The stress effect on the plants was confirmed by measuring the relative water content (RWC) and quantifying total chlorophyll. As expected, the RWC was significantly lesser (67%; $P < 0.05$) in the pots maintained at 40% FC, when compared to the pots maintained at 100% FC (85%). This reduction in water status resulted in a significant reduction in total chlorophyll content from 2.3 to 1.7 mg/g fresh weight ($P < 0.05$) in the control pots to stressed pots respectively. Drought induces chlorophyll degradation as reported in many other studies[14]. These observations indicated that the plants were experiencing drought stress and the specific level of drought stress was maintained for a period of two weeks. At the end of the stress period, the plants were subjected to total defoliation followed by full rehydration to bring the soil FC to 100%. Artificial defoliation is used to induce bud break in perennial plants[15,16]. We expected slow or delayed bud opening in stressed plants due to high levels of accumulation of inhibitors as reported in earlier studies[17]. From this context, it was expected that the control plants (maintained at 100% FC) would exhibit early bud break when compared to the stressed ones. However, an early and rapid bud break was observed in drought-stressed plants when compared to non-stressed plants (100% FC), (Figure 1). Though bud break was observed in 34% of the total buds in stressed plants, it was significantly lesser ($P = 0.01$) in control plants (11%) 10 days post defoliation and re-watering (Figure 2). Bud break increased to 32% and 51% in control and stressed plants respectively at 20 days post defoliation and re-watering. At 30 days post defoliation and rewatering, the stressed plants exhibited 54% bud break and the plants maintained at control conditions exhibited significantly lesser bud break (34%, $P = 0.01$) (Figure 2). The overall results showed an early and rapid bud break in stressed plants compared to the control plants, and such a phenomenon is not reported so far in mulberry. In India, mulberry cultivation is practised under

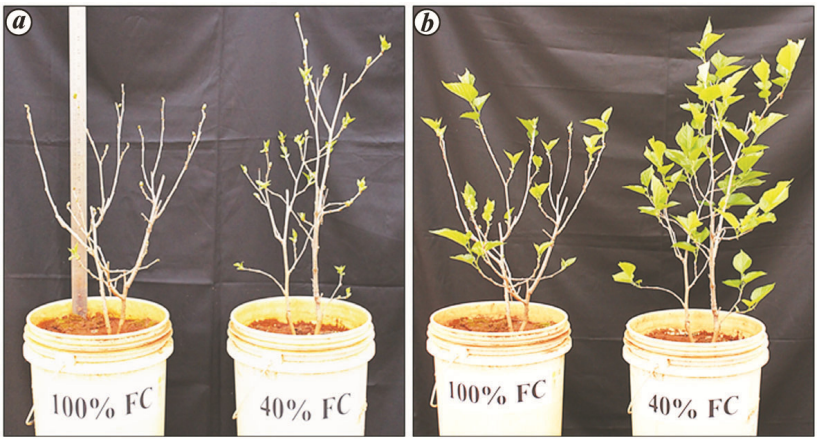

Figure 1. Representative image of bud break (***a***) 10 days of defoliation and re-watering and (***b***) 17 days after defoliation and re-watering.

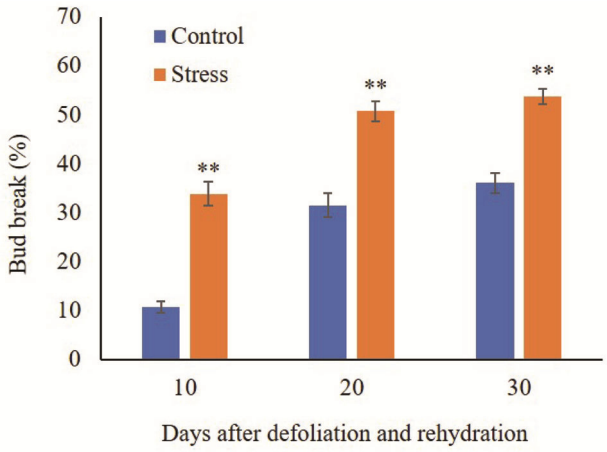

Figure 2. Bud break (%) in mulberry plants at different days after defoliation and rehydration. (Data represent the mean of six replications; error bar indicates standard error; **indicates significance at $P = 0.01$; Control – 100% soil field capacity; Stress – 40% soil field capacity).

rainfed and irrigated conditions[18]. There are two major traditional practices of harvesting foliage for silkworm rearing, one being individual leaf harvesting and the second shoot-harvesting[18]. Our finding has relevance under irrigated conditions where partial dehydration followed by hydration post leaf harvest can hasten bud growth and facilitate rapid foliage accumulation in the field. This finding opens up an opportunity for targeted experiments to induce early bud break by controlled irrigation under field conditions.

The results from the present study also put forth multiple questions to be addressed such as (i) what triggered an early and rapid bud break in stressed plants, and (ii) what are the possible mechanisms that probably alleviated repression of bud dormancy and induced early bud break? Answers to these questions require the precise examination of molecular mechanisms using multiple *omic* approaches.

1. Cooke, J. E., Eriksson, M. E. and Junttila, O., *Plant Cell Environ.*, 2012, **35**, 1707–1728.
2. Borchert, R., *Ecology*, 1994, **75**, 1437–1449.
3. Murphy, P. G. and Lugo, A. E., *Annu. Rev. Ecol. Systemat.*, 1986, **17**, 67–88.
4. Harb, A., Krishnan, A., Ambavaram, M. M. and Pereira, A., *Plant Physiol.*, 2010, **154**, 1254–1271.
5. Brunner, I., Herzog, C., Dawes, M. A., Arend, M. and Sperisen, C., *Front. Plant Sci.*, 2015, **6**, 547.
6. Lloret, A., Badenes, M. L. and Rios, G., *Front. Plant Sci.*, 2018, **9**, 1368.
7. Vimont, N. *et al.*, *bioRxiv*, 2019, p. 423871.
8. Suzuki, T., Kitano, M. and Kohno, K., *Tree Physiol.*, 1988, **4**, 53–60.
9. Suzuki, T. and Kitano, M., *Physiol. Plant.*, 1989, **75**, 188–194.
10. Dhanyalakshmi, K. H. and Nataraja, K. N., *Plant Signal. Bev.*, 2018, **13**, e1491267.
11. Guha, A., Sengupta, D., Rasineni, G. K. and Reddy, A. R., *Flora*, 2010, **205**, 144–151.
12. Sarkar, T., Mogili, T. and Sivaprasad, V., *3 Biotech*, 2017, **7**, 214.
13. Karaba, A. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 15270–15275.
14. Liu, X. *et al.*, *Sci. Rep.*, 2018, **8**, 1–11.
15. Edwards, G. R. and Notodimedjo, S., In II International Workshop on Temperate Zone Fruits in the Tropics and Subtropics, 1985, vol. 199, pp. 125–127.
16. Diaz, D. H., Alvarez, A. and Sandoval, J., In II International Workshop on Temperate Zone Fruits in the Tropics and Subtropics, 1985, vol. 199, pp. 129–136.
17. Zhao, Y., Gao, J., Im Kim, J., Chen, K., Bressan, R. A. and Zhu, J. K., *Plant Cell Physiol.*, 2017, **58**, 1319–1327.
18. Datta, R. K., *FAO Anim. Prod. Health Pap.*, 2002, **147**, 45–62.

ACKNOWLEDGEMENT. K.N.N. acknowledges the Department of Biotechnology, New Delhi for the financial support (BT/TDS/121/SP20276/2016).

Received 6 September 2019; revised accepted 2 July 2020

K. H. DHANYALAKSHMI[1]
R. S. SAJEEVAN[1,2]
K. N. NATARAJA[1,*]

[1]*Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru 560 065, India*
[2]*National Centre for Biological Sciences, TIFR, GKVK, Bengaluru 560 065, India*
**For correspondence.*
e-mail: nataraja_karaba@yahoo.com