Brief Communication

Comprehensive speed breeding: a high-throughput and rapid generation system for long-day crops

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Keywords: comprehensive speed breeding, high-throughput, light regime, canola, wheat, long-day crop, molecular breeding.

Breeding cycle time largely determines the efficiency of crop genetic improvement. To shorten the generation time, Watson et al. (2018) proposed a concept of ‘speed breeding (SB)’ by extending the photoperiod and increasing the light intensity during plant growth and achieved up to 6 and 4 generations per year for the spring wheat (Triticum aestivum) and spring canola (Brassica napus), respectively. To realize the high-throughput application of SB in combination with molecular breeding to long-day winter crops, we proposed an updated SB system designated as comprehensive SB (CSB) including vernalization of germinated seeds, high-density seedling culture, and accelerated flowering and maturation with optimized light regime. First, germinated seeds placed on wet tissue paper with visible radicles are vernalized at 4.5 °C during a 22-h light period and at 9 °C during the 2-h dark period (Figure 1a; left), with the vernalization time varying from different genotypes. Subsequently, a 96-well tray is developed for the seedling culture in a hydroponic scheme (Figure 1c), which is convenient for sample collection and genotyping in 96-well plates. Finally, the target plants are transplanted into pots and cultivated in a growth chamber, in which cold air is directed to circulate from the roof to the air flue and pass the plants, finally flowing back to the roof (Figure 1b). The light was supplied by light-emitting diode (LED) board with an optimized spectrum (Figure 1a; upper right) (Bantis et al., 2018), with the intensity of around 300 μmol/m²/s at the bench height and over 900 μmol/m²/s at 10 cm below the LED bars. The chamber is programmed to run a 22 h/2 h (light/dark) photoperiod at 22 °C with a humidity of 70% (Figure 1a; lower right). In the total growth area of 10.8 m², 2035–5400 or 675–1350 adult plants of wheat or canola can be placed with a density of 187.5–500 or 62.5–125 plants/m².

Germinated seeds of semi-winter and winter canola varieties (ZS11 and Darmor-bzh) were used to test the effect of CSB on long-day crops. After 17 days of VGS, ZS11 plants exhibited the visible flower buds at 42 days after germination (DAG) and flowered uniformly at around 48 DAG. All the siliques turned to deep yellow with hard and black seeds inside at 87 DAG (Figure 1d). Despite the rapid growth, seeds harvested from both 80 and 87 DAG plants were viable with a germination rate of more than 99% (Figure 1e). Generally, each ZS11 plant could produce more than 800 mature seeds from 30 siliques with a thousand-seed weight (5.24 ± 0.30 g, n=10), greater than that (4.57 ± 0.19 g, n=10) obtained from field conditions in Wuhan. In contrast, without vernalization, only approximately half of the plants could generate tiny flower buds at 145 DAG (Figure 1f), indicating a period of cold treatment is necessary for reducing the cycling time of semi-winter canola under the CSB conditions. CSB also functioned well for a typical spring type variety Westar, which generated the first flower at 32 DAG, finished anthesis at around 37 DAG, and produced mature seeds at 67 DAG (Figure 1g). Relative to the previous SB procedure (Hickey et al., 2019), CSB can reduce the generation time of spring type canola by 40.7% (from 113 to 67 days). Unexpectedly, despite 60 days of VGS, Darmor-bzh plants remained in the vegetative stage at 148 DAG (Figure 1h) indicating a period of cold treatment is necessary for the winter variety Yunnong19 and the spring variety Chinese Spring. After a removal of all tillers, YN19 took approximately 58 days from germination to anthesis after 30 days of VGS and gave birth to about 20 mature seeds at around 83 DAG (Figure 1i). Chinese Spring plants progressed to anthesis at about 50 DAG (without vernalization) and produced approximately 45 mature seeds at around 75 DAG, 16 days advance than that before (Watson et al., 2018) (Figure 1i). Both harvested seeds displayed normal germination rates ranging from 90% to 100%.

Since the CSB procedure failed to induce flowering of typical winter type canola variety Darmor-bzh, we further improved the light regime by adding 500 μmol/m²/s far-red light (Figure 1j). Under the new light condition with a 55-day VGS treatment, Darmor-bzh could generate visible flower buds at 92 DAG and mature seeds at around 125 DAG (Figure 1k). Aided by this improved CSB protocol, the life cycle of Westar and ZS11 could also be further accelerated by 12 and 21 days, respectively (Figure 1j; 1m). RNA sequencing of the penultimate leaf in ZS11 showed that additional far-red light in CSB significantly increased the expression of multiple activators of flowering, including the B. napus orthologues of PHYTOCHROME A, CONSTANS, and PHYTOCHROME-INTERACTING FACTOR 4 (Figure 1n). Thus, extra far-red light can collectively enhance the transcriptional levels of FLOWERING LOCUS T (FT) homologues at both stages, leading to earlier flowering than that in solo CSB.
We attempted to apply CSB in marker-assisted backcross breeding programs by introgressing a favorable haplotype of BnaA9.CYP78A9a, which can significantly increase seed weight and silique length, from ZS11 to an elite restorer 621R. As shown in Figure 1o, eight generations were accomplished within 23 months and multiple improvement lines in the BC5F3 families were obtained. Among them, 621R-A9 exhibited a 97.7% background recovery rate of the recipient genome as revealed by whole-genome re-sequencing (Figure 1p). Field evaluation showed that the thousand-seed weight and silique length of 621R-A9 were significantly higher than those of 621R (Figure 1q). Interestingly, some other yield and quality-related traits were also...
significantly optimized relative to the recipient (Figure 1q). These results indicate that CSB is effective and time-saving for molecular breeding in semi-winter canola.

In summary, we propose a CSB system for the high-throughput culture and rapid generation of long-day crops. Application of CSB can cycle 4.5 and 5.5 generations per year for semi-winter and spring canola, respectively. Complementation of extra far-red light to CSB not only helps to reproduce winter canola for 3 generations per year but also further accelerate one more generation for other type canola. Moreover, about 4.5 to 5 generations in 1 year can be accomplished for both spring and winter wheat under CSB. This strategy is expected to greatly accelerate gene pyramiding of superior alleles, screening of recombinants for QTL mapping and functional genomics research by rapid purification of multiple mutated alleles.

Acknowledgements

This research was supported by Natural Science Foundation of Hubei Province (2019CFA090), Wuhan Applied Foundational Frontier Project (201902070111446), and the Open Funds of the National Key Laboratory of Crop Genetic Improvement (ZK201909).

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

D.H. and G.Y. designed the project. Y.S, X.D., P.W., X.Y., and Z.W. performed the experiments. Y.S. wrote the manuscript with the help of L.W. and X.L.

References

Bantis, F., Smirnakou, S., Ouzounis, T., Koukounaras, A., Ntagkas, N. and Radoglou, K. (2018) Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). *Sci. Hortic.* 235, 437–451.

Hickey, L.T., N. Hafeez, A., Robinson, H., Jackson, S.A., Leal-Bertioli, S.C.M., Tester, M., Gao, C. et al. (2019) Breeding crops to feed 10 billion. *Nat. Biotechnol.* 37, 744–754.

Watson, A., Ghosh, S., Williams, M.J., Cuddy, W.S., Simmonds, J., Rey, M.-D., Asyraf Md Hatta, M. et al. (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. *Nat. Plants*, 4, 23–29.