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# *Brachypodium distachyon* is a suitable host plant for study of Barley yellow dwarf virus

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**Abstract** Barley yellow dwarf viruses (BYDVs) belong to the family *Luteoviridae* and cause disease in cereals. Because of the large and complex genome of cereal plants, it is difficult to study host-virus interactions. In order to establish a model host system for the studies on BYDVs, we examined the susceptibility of a monocot model plant, *Brachypodium distachyon*, to BYDV-GAV infection. Fourteen days after BYDV-GAV inoculation by aphid transmission, *B. distachyon* plants (inbred line Bd21-3) showed conspicuous disease symptoms such as leaf reddening, dwarfness and root stunting. Virus accumulation was detected in both shoots and roots using reverse transcription PCR and triple antibody sandwich ELISA. Compared with infected wheat plants, *B. distachyon* plants developed more severe disease symptoms and accumulated a higher level of BYDV-GAV. Under transmission electron microscope, we observed that virus particles accumulated in companion cells and BYDV-GAV infection was associated with the deformation of chloroplasts in the infected leaves of *B. distachyon* plants. Our results suggest that *B. distachyon* is a suitable and promising experimental model plant for the host-BYDV-GAV pathosystem and possibly for other BYDVs.

**Keywords** Barley yellow dwarf virus · *Brachypodium distachyon* · Model plant · Virus disease · Virus-host pathosystem

Important agricultural monocot crops such as wheat, barley and rice are typically infected by numerous viruses. Because plant viral diseases are difficult to control, a better understanding of plant-virus interactions might facilitate the development of novel strategies for effective control. However, compared with model plants, cereal crops containing large genomes and duplicated genes are more difficult to study; due to that, limited methods are available for gene functional analysis and genetic manipulation of virus genes. The establishment of a pathosystem using a model plant will facilitate studies on the molecular interactions between cereal viruses and their host plant species.

*Brachypodium distachyon*, a temperate monocot (genus *Pooideae*, family *Poaceae*), is an emerging model plant for the functional genomics study of temperate grasses and cereals owing to its small size, simple genome, short life cycle and undemanding growth requirements [1]. *B. distachyon* possesses agronomic traits that are in common with temperate cereals (wheat, barley and oat) [2]. The sequencing of full genome of *B. distachyon* accession Bd21 was completed by the International Brachypodium Initiative (IBI) in 2010. Genomics analysis revealed that *B. distachyon* shares high degrees of chromosomal synteny with wheat and barley plants [3]. A stable transformation system mediated by *Agrobacterium tumefaciens* was developed [4] and thousands of insertion lines have been generated and assigned to unique locations in the genome

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[5]. Furthermore, virus-induced gene silencing using *Barley stripe mosaic virus* (BSMV) was reported to be effective in *B. distachyon* [6]. Therefore, *B. distachyon* is a promising model plant for the exploration of cereal-pathogen interactions. However, other than in BSMV [7], the potency of *B. distachyon* as a model host for cereal viruses pathosystem has not been studied extensively.

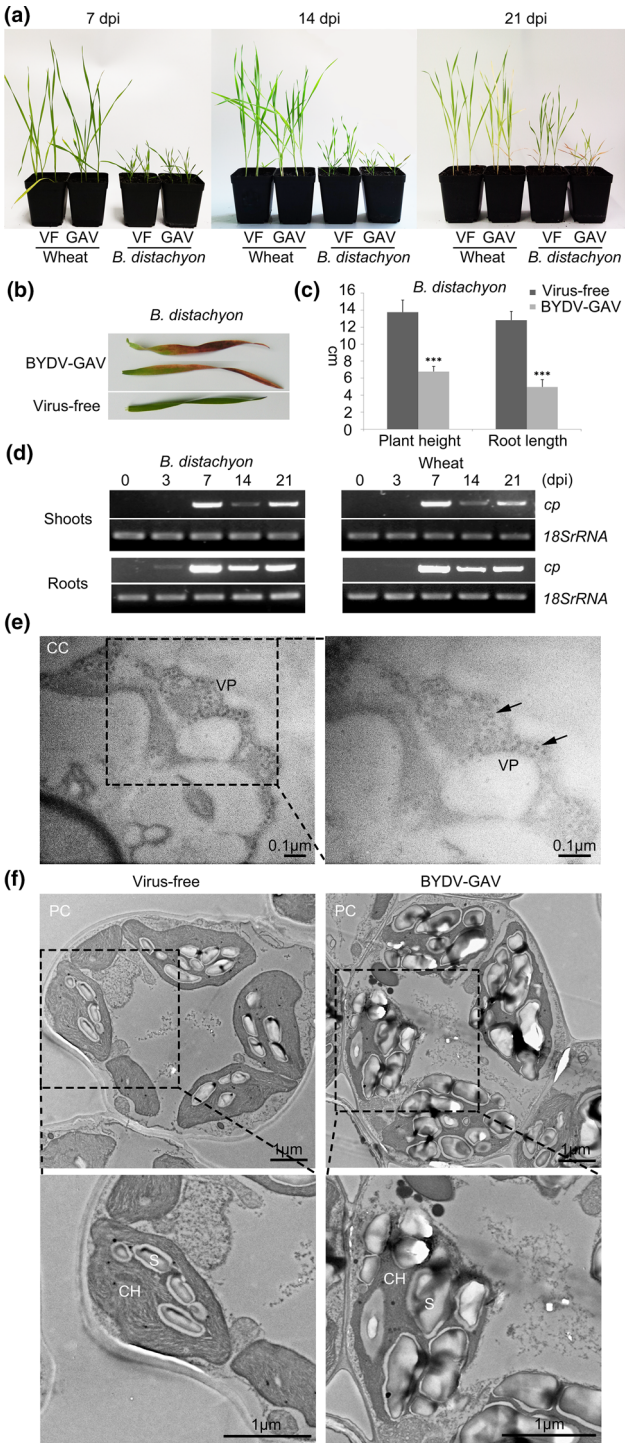
Yellow dwarf disease in cereal plants including barley, wheat and oat, is considered to be one of the most important viral diseases worldwide because it significantly reduces the production and quality of cereals. The disease caused by *Barley yellow dwarf viruses* (BYDVs) shows typically characteristic symptoms, such as foliar yellowing, reddening and plant stunting. BYDVs belong to the genus *Luteovirus* in the family *Luteoviridae* and the viruses consist of approximately 30 nm icosahedral capsid proteins (CPs). BYDVs are solely transmitted to host plants by cereal aphids species, such as *Rhopalosiphum padi*, *Schizaphis graminum* and *Sitobion avenae* in nature [8]. BYDVs consist of BYDV-PAV, BYDV-PAS, BYDV-MAV, BYDV-kerII and BYDV-kerIII in the genus *Luteovirus*, and BYDV-GPV, BYDV-SGV and BYDV-GAV, which are not yet assigned to a genus (ICTV, 2014). BYDV-GAV was isolated from China and was demonstrated to have a serological relationship with BYDV-MAV [9]. In China, wheat is considered to be the most important host of BYDVs because of its widespread cultivation. Given the complex genome and difficulty in genetic manipulation of wheat, it is necessary to establish a host model plant for effective study on the interaction between BYDVs and host plants.

To determine the efficacy using *B. distachyon* as an experimental host for BYDVs, we inoculated BYDV-GAV isolate 05YL6 (Accession No. EU402386) into *B. distachyon* (inbred line Bd21-3) via aphid vector (*Schizaphis graminum*) transmission under laboratory condition as described previously [10]. In addition, a wheat cultivar (line 7182) susceptible to BYDV-GAV was used as a positive control for evaluation of virus accumulation and infectivity in the same experiments. Before inoculation, the aphids were transferred to BYDV-GAV-infected *Avena sativa* cv. Coast Black plants for 3 days to acquire the virus. Each seedling (at 2–3 leaf stage) was inoculated with 5 viruliferous aphids and then covered by plastic tubes to restrict them. Three days later, the aphids were killed by insecticide, and the plants were placed in the greenhouse with a cycle of 16 h-light (22 °C) and 8 h-dark (18 °C) and a relative humidity of 65 %. Total sixty plants (30 *B. distachyon* and 30 wheat) were examined at one time, and the experiment was repeated three times. At 7 days post inoculation (dpi), neither *B. distachyon* nor wheat plants showed any virus disease symptoms (Fig. 1a). At 14 dpi, the typical viral disease symptoms of dwarfing and leaf reddening were observed in *B. distachyon* but no viral symptom was observed in the wheat plants (Fig. 1a). At 21 dpi, *B. distachyon* plants showed more serious symptoms of shortening of roots and leaf necrosis (Fig. 1b, c). All inoculated wheat and *B. distachyon* plants showed disease symptoms, indicating high efficiency of BYDV-GAV infection in both plant hosts. However, wheat plants only showed yellowing symptoms on leaves but no dwarfing symptom was observed, while most *B. distachyon* plants exhibited leaf reddening and the plants grew significantly smaller than the healthy ones (Fig. 1a). This observation showed that symptoms of BYDV-GAV appear faster and more severe in *B. distachyon* than in wheat plants. Virus accumulation in shoots (leaves and stem tissues) and roots was also determined by semi-quantitative RT-PCR using a primer set CP-F (5′-ATGAATTCAG TAGGCCGTAGAAAT-3′) and CP-R (5′-CTATTTG GGAGTCATGTTGGCAAC-3′) specific for the coat protein gene. In parallel, *18SrRNA* gene served as a reference gene [11]. The results indicated that BYDV-GAV accumulation was detected in the roots of *B. distachyon* plants as early as 3 dpi and systemically spreads to the whole plants from 7 dpi (Fig. 1d).

To analyse BYDV-GAV titer in *B. distachyon* and wheat plants, triple antibody sandwich (TAS)-ELISA using an antibody specific for BYDV-MAV virions was carried out as described previously [10]. The results indicated that in both host plants, BYDV-GAV titer was significantly increased at 7 dpi (seven-fold; $P < 0.05$, Table 1) but then reduced at 14 and 21 dpi (Table 1). TAS-ELISA also showed that at 21 dpi, BYDV-GAV titer was higher in *B. distachyon* than wheat plants (Table 1). This result is coincident with the observation that *B. distachyon* showed more severe symptoms than wheat plants (Fig. 1a).

To examine the intracellular distribution and cytopathological effects of BYDV-GAV on *B. distachyon*, ultrathin sections were prepared from infected and non-infected leaves as described previously [12]. Under transmission electron microscope (TEM), we observed that BYDV-GAV virions exclusively accumulated in companion cells (Fig. 1e). In parenchymal cells, BYDV-GAV infection resulted in the deformation of chloroplasts, in which the starch grains became swollen and the grana and stroma lamellae were disrupted (Fig. 1f). In contrast, the chloroplasts of non-infected plants had a regular shape and well-organised grana and stroma lamellae (Fig. 1f).

To further explore the potential of *B. distachyon* as a BYDV-GAV host, a simple aphid-mediated transmission of BYDV-GAV between *B. distachyon* plants was conducted. The virus was acquired from BYDV-GAV-infected *B. distachyon* plants and 15 seedlings were inoculated as described above. At 21 dpi, they exhibited similar symptoms with *B. distachyon* plants infected by BYDV-GAV




◀**Fig. 1** Symptoms and accumulation of BYDV-GAV in *B. distachyon* and wheat plants. **a** Development of viral symptoms in *B. distachyon* and wheat plants. Five viruliferous aphids were inoculated to each plant and fed for three days. VF, virus-free plants; GAV, BYDV-GAV-infected plants. **b** Leaf reddening symptom in *B. distachyon* plants. Leaves (the third position from *top*) were photographed at 21 dpi. **c** Reduction of plant height and length of root in *B. distachyon* plants. Eight infected and six virus-free *B. distachyon* plants were measured at 21 dpi in one experiment. The data shown are representative of three independent experiments. Independent *t* test was used for data analysis. $***P < 0.001$. **d** Semi-quantitative RT-PCR analysis of BYDV-GAV accumulation in different plant tissues. The shoots and roots of three *B. distachyon* and wheat, respectively, were sampled at 0, 3, 7, 14, 21 dpi. RNA extracted from the tissues was subjected to semi-quantitative RT-PCR amplification (28 cycles) with the gene-specific oligonucleotide primers CP-F and CP-R. Amplified *18S rRNA* of *B. distachyon* and wheat served as an internal control. **e** Transmission electron micrographs of virus particles in companion cells. The ultrathin sections were prepared from the newly emerged leaves of virus-infected *B. distachyon* plants. Arrows indicate the particles of BYDV-GAV. The box to the left represents an enlargement of the boxed area to the right. CC, companion cell; VP, virus particles. **f** Transmission electron micrographs of chloroplasts in parenchymal cells of leaf tissues. The ultrathin sections were prepared from the newly emerged leaves of non-infected and virus-infected *B. distachyon* plants. The box above represents an enlargement of the boxed area below. PC, parenchymal cells; CH, chloroplasts; S, starch grains

from oats (Supplemental Fig. 1), indicating the virus could spread in *B. distachyon* plants through aphid-mediated transmission. The systemic infection was also confirmed by TAS-ELISA and RT-PCR as described above (data not shown).

BYDVs are phloem-limited viruses, causing damage to the phloem and its associated plasmodesmata [13]. BYDVs infection is thought to restrict the transport of photosynthates [14]. Disease symptoms shown by different cereals to BYDVs vary. BYDVs mainly cause leaf yellowing and sometimes plant stunting in wheat and barley plants, while they cause more severe symptoms in oats, such as yellowing, reddening or stiffness of leaves, reduced tillering and heading and numerous sterile florets [15]. Compared with the three main hosts of BYDVs, symptoms caused by BYDV-GAV in *B. distachyon* are highly similar to those in oats but have little resemblances with those in wheat and barley plants. In oat plants infected with BYDVs, leaf blade yellowing or reddening symptoms appear first on older leaves and then often progress into necrosis [16].These symptom phenotypes were also observed in *B. distachyon* infected with BYDV-GAV (Fig. 1a, b). The older leaves of *B. distachyon* first showed reddening at 14 dpi and then exhibited visible necrosis at 21 dpi. The dwarfing symptom induced by BYDVs was not always observed in wheat and barley plants [15], whereas it was consistently observed in all of the inoculated *B. distachyon* plants (Fig. 1a). In this study, the susceptible wheat cultivar line 7182 also showed the leaf yellowing symptoms but no shoots stunting at 21 dpi (Fig. 1a). BYDVs-infected cereal plants had approximately 40 % reduction in total root length [17], which is possibly caused by large reduction in carbohydrate translocation from source leaves to root through the phloem [13]. Similarly, BYDV-GAV-infected *B. distachyon* also showed root stunting (Fig. 1c). In addition, BYDV-GAV infection induced deformation of chloroplast structures (Fig. 1f),

**Table 1** Comparison of virus accumulations between *B. distachyon* and wheat plants

| Host | 0 dpi | | 3 dpi | | 7 dpi | | 14 dpi | | 21 dpi | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Symptoms | ELISA[a] | Symptoms | ELISA[a] | Symptoms | ELISA[a] | Symptoms | ELISA[a] | Symptoms | ELISA[a] |
| Wheat | – | 0.198c[b] | – | 0.479c | – | 3.873a | – | 1.227b | Leaf yellowing | 1.228b |
| Mock-W | – | 0.197a | – | 0.206a | – | 0.221a | – | 0.210a | – | 0.209a |
| *B. distachyon* | – | 0.239d | – | 0.486d | – | 3.386a | Dwarfing and leaf reddening | 1.384c | Dwarfing and leaf reddening | 2.120b |
| Mock-Bd | – | 0.248a | – | 0.234a | – | 0.212[a] | – | 0.233a | – | 0.210a |

[a] Absorbance values at 405 nm. Three plants (both of shoots and roots) of mock and infected *B. distachyon* and wheat, respectively, were sampled at 0, 3, 7, 14, 21 dpi and analysed by TAS-ELISA in one experiment. The data shown are representative of two independent experiments

[b] Within lines, means followed by the same letter are not significantly different at $P < 0.05$. Turkey HSD was used for mean separation

*dpi* days post inoculation, *Mock-W* virus-free wheat plants, *Mock-Bd* virus-free *B. distachyon* plants

which is identical to that previously observed in wheat plants [18].

Though symptoms of *B. distachyon* produced by BYDV-GAV are more distinct than those of wheat plants, BYDV-GAV showed a relatively similar accumulation pattern in both host plants (Fig. 1d and Table 1). Little of BYDV-GAV was detectable in the roots of *B. distachyon* plants at 3 dpi, while the virion titer reached the highest level at 7 dpi, indicating a rapid increased accumulation of BYDV-GAV from 3 dpi to 7 dpi. Though the virus titer decreased after 7 dpi in both wheat and *B. distachyon* plants, viral symptoms appeared at the later infection stage, indicating that a high level of BYDV-GAV accumulation precedes the emergence of disease symptoms and that the viral symptoms are not strictly linked with the level of BYDV-GAV titer. Nevertheless, at 21dpi, BYDV-GAV titer was higher in *B. distachyon* than wheat plants. This may be in accordance with more severe viral symptoms in *B. distachyon* than wheat plants (Fig. 1d and Table 1).

In summary, this study demonstrated that *B. distachyon* is highly susceptible to BYDV-GAV infection and displayed more severe disease symptoms than wheat. Similar cytological changes and pattern of virus accumulation with wheat were also observed in *B. distachyon* plants. Hence, *B. distachyon* is an excellent model host plant for BYDV-GAV and will facilitate the deeper studies to unravel the molecular aspects of BYDVs-hosts interactions.

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