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DISEASE NOTE

First report of barley virus G infecting winter barley (*Hordeum vulgare* L.) in France

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1 As part of a cereals virome project high throughput sequencing (HTS)-based viral indexing was
2 performed on plants of various cereals with symptoms of barley yellow dwarf disease collected
3 in June (2017-2020) in the main French cereals production areas. Total RNAs from 32
4 individual plants, including 9 winter barley plants were purified (RNeasy Plant Mini Kit,
5 Qiagen, Courtaboeuf, France) and Illumina sequenced (2x150 nt) following ribodepletion
6 (Genewiz-Azenta, Leipzig, Germany). Following quality trimming, reads for each sample were
7 *de novo* assembled (CLC Genomics Workbench 21, Qiagen) [1] and contigs annotated by
8 BlastX analysis. In four winter barley samples collected in 2018 (18-58, 18-325 and 18-326)
9 and 2019 (19-30A), besides contigs representing diverse viruses such as barley yellow dwarf
10 viruses-PAV and PAS, *Hordeum vulgare* endornavirus, cereal yellow dwarf virus-RPV (18-
11 326), wheat dwarf virus (18-325 and 18-326) and a novel *Polerovirus* (18-58 and 18-326),
12 large contigs with high identity to barley virus G (BVG) were identified. BVG, a tentative
13 *Polerovirus*, was initially reported in barley in South Korea in 2016 [2] and has so far been
14 identified in a few other hosts including wheat, oat, maize, proso and foxtail millets as well as
15 switchgrass. It has been reported from the USA, Australia [3] and, in Europe, from the
16 Netherlands, Germany, Hungary and Greece [4]. Large BVG scaffolds representing near
17 complete genomes could be reconstructed for each sample, integrating a total of 128.339,
18 7.188, 8.078 and 20.073 reads, for samples 19-30A, 18-58, 18325 and 18-326 respectively.
19 Given that between 17.2 and 20.5 million reads had been obtained per sample, these values
20 translate into between 0.04% (18-58 and 18-325) and 0.6% (19-30A) of total reads, and to
21 average coverages of between 158x (18-58) and 2866x (19-30A) for the genomic scaffolds.
22 The four assembled sequences (5584-5610 nt) have been deposited in GenBank (ON419453-
23 ON419456). They are nearly identical (98.4 to 99.5% nt identity) and share between 97.7% and
24 98.5% nt identity with a barley reference isolate from the South Korea (NC_029906). To
25 confirm the presence of BVG, a primer pair was designed based on available BVG sequences.

26 Primers BVG-F(5'-CTAGCCCAACGAGTTGCGGG-3') and BVG-R(5'-
27 GGTACAGAAGCTCTACGGTTC-3') amplifying a 394 nt product were used in a two-step
28 RT-PCR on new RNA extracts obtained from the 18-325 and 18-326 infected plants. The
29 amplicons were directly sequenced and showed respectively 99.2% (ON419457, 18-325) and
30 100% (18-326) nt identity with the corresponding *de novo* scaffolds. The four analyzed samples
31 have been collected respectively in 2018 (18-58, 18-325, 18-326) and 2019 (19-30A) in three
32 different regions of France (Auvergne-Rhône-Alpes, Occitanie and Centre-Val de Loire),
33 indicating a wide distribution and a persistence over time of BVG in France. To our
34 knowledge, this represents the first report of a natural infection of BVG in cultivated winter
35 barley in France. Presence of BVG may have been overlooked in a range of situations, as
36 indicated by its retrospective discovery in a 34 years old Australian sample [3], possibly
37 explaining its broad distribution in France. While the mixed infection status of the analyzed
38 plants precludes any conclusion on its pathogenicity in French cereals, BVG has been reported
39 to be associated with a range of symptoms in various hosts so that further studies to evaluate its
40 prevalence and impact in France and to begin to understand its epidemiology are clearly
41 warranted by the present results.

42 **References**

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