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| R2  S  *E.c.* | 1641 1661 1681  |......... |......... |......... |......... |......... |.........  .........DIGMVAWILE MSTPEFPNGR QIIVVANDIT FRAGSFGPRE DAFFEAVTNM ACEKKLPLIY  .........DIGMVAWILE MSTPEFPNGR QIIVVANDIT FRAGSFGPRE DAFFEAVTNM ACEKKLPLIY  .........DIGMVAWILD MSTPEFPSGR QIIVIANDIT FRAGSFGPRE DAFFEAVTNL ACEKKLPLIY  \*\*\*\*\*^\*\*^$ \*\*\*\*\*^\*$\*^ \*\*\*^$\*\*\*\*^ ^\*\*\*\*\*\*\*\*\* \*\*\*^\*\*^\*\*$ ^^\*\*\*\*^\*^\* |
| R2  S  *E.c.* | 1701 1721 1741 **Gln-1756** 1761  |......... |......... |......... |......... |......... |......... |.........  LAANSGARIG IADEVKSCFR VGWSDEGSPE RGFQYIYLTE EDYARISSSV IAHKL**Q**LDSG EIRWIIDSVV  LAANSGARIG IADEVKSCFR VGWSDEGSPE RGFQYIYLTE EDYARISSSV IAHKL**Q**LDSG EIRWIIDSVV  LAANSGARIG IADEVKSCFR VGWTDDSSPE RGFRYIYMTE EDYDRISSSV IAHKV**Q**LDSG EVRWIIDSVV  \*^\*\*\*\*\*^\*\* ^\*\*\*^\*\*\*\*\* \*^\*$\*$$\*\*\* \*^\*$\*\*\*$\*\* \*\*\*$\*\*\*\*^\* \*\*\*\*$\*^\*\*\* ^^\*\*$\*^\*\*\* |
| R2  S  *E.c.* | **Ile-1781** 1801 1821  |......... |......... |......... |......... |......... |......... |.........  GKEDGLGVEN **I**HGSAAIASA YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK  GKEDGLGVEN **I**HGSAAIASA YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK  GKEDGLGVEN **I**HGSAAIASA YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK  ^^\*\*^^\*^\*^ \*\*\*\*\*\*\*\*\*^ \*\*\*^^\*\*\*\*\* \*\*^^\*^^\*^\* ^\*^\*\*\*^\*^\* ^\*\*^\*^\*^^\* \*^^\*\*\*^^\*\* |
| R2  S  *E.c.* | 1841 1861 1881 1901  |......... |......... |......... |......... |......... |......... |.........  LLGREVYSSH MQLGGPKIMA TNGVVHLTVS DDLEGVSNIL RWLSYVPANI GGPLPITKPL DPPDRPVTYI  LLGREVYSSH MQLGGPKIMA TNGVVHLTVS DDLEGVSNIL RWLSYVPANI GGPLPITKPL DPPDRPVTYI  LLGREVYSSH MQLGGPKIMA TNGVVHLTVP DDLEGVSNIL RWLSYVPANI GGPLPITKSL DPIDRPVAYI  \*\*^\*^\*\*\*\*\* \*^\*\*\*^^^\*\* ^\*\*\*\*^^\*\*$ \*^\*\*\*\*^\*\*\* \*\*\*\*\*\*\*^\*\* \*\*\*\*\*\*\*\*$\* \*\*$\*\*^\*$^^ |
| R2  S  *E.c.* | 1921 1941 1961  |......... |......... |......... |......... |......... |......... |.........  PENTCDPRAA IRGVDDSQGK WLGGMFDKDS FVETFEGWAK TVVTGRAKLG GIPVGVIAVE TQTMMQLIPA  PENTCDPRAA IRGVDDSQGA WVGGMFDKDS FVETFEGWAK TVVTGRAKLG GIPVGVIAVE TQTMMQLIPA  PENTCDPRAA ISGIDDSQGK WLGGMFDKDS FVETFEGWAK TVVTGRAKLG GIPVGVIAVE TQTMMQLVPA  \*\*^\*\*\*^\*\*\* ^$^$\*\*\*\*\*\* \*\*\*^\*\*\*\*\*^ \*\*\*\*\*\*\*\*\*^ \*^\*\*^\*\*^\*^ ^\*\*^\*^\*\*\*\* \*^\*\*\*\*^$\*\* |
| R2  S  *E.c.* | 1981 **Try-1999** 2001 2021 **Trp-2027 Ile-**2041  |......... |......... |......... |......... |......... |......... |.........  DPGQLDSHER SVPRAGQV**W**F PDSATKTAQA LLDFNREGLP LFILAN**W**RGF SGGQRDLFEG **I**LQAGSTIVE  DPGQLDSHER SVPRAGQV**W**F PDSATKTAQA LLDFNREGLP LFILAN**W**RGF SGGQRDLFEG **I**LQAGSTIVE  DPGQPDSHER SVPRAGQV**W**F PDSATKTAQA MLDFNREGLP LFILAN**W**RGF SGGQRDLFEG **I**LQAGSTIVE  \*\*\*\*$\*\*^\*\* \*\*\*^\*^\*^\*\* \*\*\*^\*\*\*^\*^ $\*^\*\*\*\*\*^\* ^\*^\*\*\*\*\*^\* \*^^\*\*\*^\*\*\* \*^\*\*\*\*\*\*\*\*\* |
| R2  S  *E.c.* | 2061 **Asp-2078** 2081 **Cys-2088** **Gly-2096** 3001  |......... |......... |......... |......... |......... |......... |.........  NLRTYNQPAF VYIPMAGELR GGAWVVV**D**SK INPDRIE**C**YA ERTAK**G**NVLE PQGLIEIKFR SEELQDCMGR  NLRTYNQPAF VYIPMAGELR GGAWVVV**D**SK INPDRIE**C**YA ERTAK**G**NVLE PQGLIEIKFR SEELQDCMGR NLRTYNQPAF VYIPKAAELR GGAWVVI**D**SK INPDRIE**C**YA ERTAK**G**NVLE PQGLIEIKFR SEELKECMGR  \*\*\*\*\*\*\*\*^\* ^^^^$\*$\*^\* \*\*^\*^\*$\*\*^ \*^\*^\*^\*^\*\* \*\*\*\*^^\*^^\* \*\*\*^\*^\*\*\*^ \*\*\*\*$$^\*^\* |
| R2  S  *E.c.* | 3021 3041 3061  |......... |......... |......... |......... |......... |.........  LDPELINLKA KLQGAKLGNG SLPDIESLQK SIEARTKQLL PLYTQIAIRF AELHDTSLRM.........  LDPELINLKA KLQGAKLGNG SLPDIESLQK SIEARTKQLL PLYTQIAIRF AELHDTSLRM.........  LDPELIDLKA RLQGAKLGNG SLSDGESLQK SIEARKKQLL PLYTQIAVRF AELHDTSLRM.........  \*^\*^\*\*$\*\*\* $\*^^\*^\*\*\*\* ^\*$\*$\*\*\*\*\* ^\*\*\*^$\*\*\*^ \*^^\*^\*^$\*\* ^\*\*^^\*\*^\*\* |
| **Supplementary data 1:** Sequence alignment of plastid ACCase gene in the carboxyltransferase domain from the R2, S, *E.c..* The amino acids, which were identical marked with asterick (\*) identity. Silent codons in amino acid were underlined and marked by using ‘^’ , and missense codons in amino acid were underlined and marked with ‘$’.Abbreviations: R2, Resistant biotype2; S, Susceptible biotype; *E.c.*, *E. crus-galli* (accession number: KU198448) | |

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| 1741 1751 1761  |.............. |.............. |.............. |.............. |.. |
| Mutant GAAGACTATG**C**TCGT ATTAGCTCTTCTGTT ATAGCACACAAG**C**TG CAGCTGGATAGTGGT GAA  Wildtype XXXGACTATG**C**TCGT ATTAGCTCTTCTGTT ATAGCACACAAG**C**TG CAGCTGGATAGTGGT GAA  S XXXGACTATG**C**TCGT ATTAGCTCTTCTGTT ATAGCACACAAG**C**TG CAGCTGGATAGTGGT GAA  *E.c.* GAAGACTATG**A**TCGT ATTAGCTCTTCTGTT ATAGCACACAAG**G**TG CAGCTGGATAGTGGT GAA  $ $  1771 **Ile-1781-Leu**  ............ |.............. |.............. |.............. |..... |
| Mutant **A**TTAGGTGGATT ATTGACTCTGTTGTG GGCAAGGAGGATGG**G** CTTGGTGTTGAGAAT **C**TACAT  Wildtype **A**TTAGGTGGATT ATTGACTCTGTTGTG GGCAAGGAGGATGG**G** CTTGGTGTTGAGAAT **A**TACAT  S **A**TTAGGTGGATT ATTGACTCTGTTGTG GGCAAGGAGGATGG**G** CTTGGTGTTGAGAAT **A**TACAT  *E.c.* **G**TTAGGTGGATT ATTGACTCTGTTGTG GGCAAGGAGGATGGT CTTGGTGTTGAGAAT **A**TACAT  $ \*  1791 1801  ......... |.............. |.............. |.............. |........ |
| Mutant GGAAGTGCT GCTATTGCCAGTGCT TATTCTAGGGCATAT GAGGAGACATTTACA CTTACATTC  Wildtype GGAAGTGCT GCTATTGCCAGTGCT TATTCTAGGGCATAT GAGGAGACATTTACA CTTACATTC  S GGAAGTGCT GCTATTGCCAGTGCT TATTCTAGGGCATAT GAGGAGACATTTACA CTTACATTC  *E.c.* GGAAGTGCT GCTATTGCCAGTGCT TATTCTAGGGCATAT GAGGAGACATTTACA CTTACATTC  1811 1821  ...... |.............. |.............. |.............. |........... |
| Mutant GT**A**ACT GG**G**CGGACTGTAGGA ATAGGAGCTTATCTT GCTCGGCTTGGTAT**C** CG**T**TGCATACA**A**  Wildtype GT**A**ACT GG**G**CGGACTGTAGGA ATAGGAGCTTATCTT GCTCGGCTTGGTAT**C** CG**T**TGCATACA**A**  S GT**A**ACT GG**G**CGGACTGTAGGA ATAGGAGCTTATCTT GCTCCGCTTGGTAT**C** CG**T**TGCATACA**A**  *E.c.* GT**G**ACT GG**A**CGGACTGTAGGA ATAGGAGCTTATCTT GCTCGGCTTGGTAT**A** CG**G**TGCATACA**G**  1831 1841  ... |.............. |.............. |.............. |................ |
| Mutant CGT CT**T**GACCA**A**CCTATT ATTTTAAC**A**GGGTTT TCTGC**T**CT**A**AACAAG CTTCTTGGGXXXXXXXXX  Wildtype CGT CT**T**GACCA**A**CCTATT ATTTTAAC**A**GGGTTT TCTGC**T**CT**A**AACAAG CTTCTTGGACGGGAAGTG  S CGT CT**T**GACCA**A**CCTATT ATTTTAAC**A**GGGTTT TCTGC**T**CT**A**AACAAG CTTCTTGGTCGGGAAGTG  *E.c.* CGT CT**A**GACCA**G**CCTATT ATTTTAAC**T**GGGTTT TCTGC**C**CT**G**AACAAG CTTCTTGGGCGGGAAGTG |
| **Supplementary data 2:** Nucleotide sequences of mutanted and wild type cDNA fragments surrounding the Ile1781 to Leu substitution in resistant biotype, R1. Sequences were aligned with susceptible biotype (S), and *E. crus-galli* (*E.c.*) (accession number: KU198448). Silent codons were bolded and underlined, missense codons were bolded, underlined and marked by using ‘$’, and amino acid substitution (ATA to CTA) were bolded, underlined and marked with ‘\*’. |

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| 174117511761 1771 **Ile-1781-Leu**  .... |.......... |........... |.......... |.......... |.......... |
| Mutant ..TE EDYARISSSV IAHKLQLDSG EIRWIIDSVV GKEDGLGVEN **L**HGSAAIASA  Wildtype ..XX XDYARISSSV IAHKLQLDSG EIRWIIDSVV GKEDGLGVEN **I**HGSAAIASA  S ..XX XDYARISSSV IAHKLQLDSG EIRWIIDSVV GKEDGLGVEN **I**HGSAAIASA  *E.c.* ..TE EDYDRISSSV IAHKVQLDSG EVRWIIDSVV GKEDGLGVEN **I**HGSAAIASA  $ $ $ \*  1791 1801 1811 1821 1831 1841  |.......... |.......... |.......... |.......... |.......... |........ |
| Mutant YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK LLGXXX..  Wildtype YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK LLGREV..  S YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK LLGREV..  *E.c.* YSRAYEETFT LTFVTGRTVG IGAYLARLGI RCIQRLDQPI ILTGFSALNK LLGREV.. |
| **Supplementary data 3.** Amino acid sequences of mutanted and non-transgenic wild type cDNA fragments of resistant biotype, R1. Transcribed amino acid sequences were aligned with susceptible biotype (S) and *E. crus-galli* (*E.c.*) (accession number: KU198448). Silent codons in amino acids were underlined. Missense codons, which were presented in both susceptible and resistant biotypes underlined and marked by using ‘$’. The amino acid substitution lle-1781 to Leu were bolded, underlined and marked with ‘\*’. |

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| **Amino acid**  **position** | **Frequency**  **(Same reference)** | **Frequency**  **(Different reference)** | **Codon** | **Amino acid substitution** |
| 1456 | 68.48 | 31.52 | AAT/AAC | N(Asn) |
| 1516 | 66.32 | 33.33 | AGA/AGG | R (Arg) |
| 1543 | 75.65 | 24.36 | TGC/TGT | C (Cys) |
| 1555 | 75.06 | 24.94 | TTG/TTA | L (Leu) |
| 1705 | 75.18 | 24.82 | TCT/TCC | S(Ser) |
| **1781** | **60.21** | **39.58** | **ATA/CTA** | **I/L(lle/Leu)** |
| 1849 | 60.17 | 39.62 | TTC/TTT | F(Phe) |
| 1852 | 68.38 | 31.62 | GTA/GTG | V(Val) |
| 1900 | 58.67 | 41.09 | ATC/ATA | I (Ile) |
| 1903 | 70.07 | 29.93 | CGT/CGG | R(Arg) |
| 1969 | 76.66 | 22.71 | GGT/GGA | G(Gly) |
| 1981 | 69.95 | 30.05 | TAC/TAT | Y(Tyr) |
| 2023 | 70.87 | 29.13 | ACC/ACT | T(Thr) |
| 2293 | 72.8 | 26.99 | ACA/ACT | T(Thr) |
| 2311 | 74.25 | 25.75 | GCA/GCT | A(Ala) |
| 2323 | 55.75 | 43.65 | GGT/GGA | G(Gly) |
| 2374 | 73.56 | 26.27 | ATC/ATT | I(Ile) |
| 2431 | 67.52 | 32.48 | CAA/CAG | Q(Gln) |
| 2474 | 67.54 | 32.46 | TTG/CTG | L(Leu) |
| 2549 | 60.12 | 39.56 | CTG/TTG | L(Leu) |
| 2593 | 76.83 | 23.17 | AAC/AAT | N(Asn) |
| 2617 | 73.08 | 26.37 | GCA/GCG | A(Ala) |
| 2650 | 70.96 | 28.77 | CGT/CGC | R(Arg) |
| 2719 | 75.17 | 24.36 | ACT/ACC | T(Thr) |
| 2932 | 76.23 | 23.77 | ACC/ACG | T(Thr) |
| 2971 | 73.91 | 26.09 | TCC/TCT | S(Ser) |
| Average | 69.75% | 30.09% |  |  |
| **Supplementary data 4**: Single nucleotide variations of *D. ciliaris* ACCase gene in both resistant biotypes, R1 and R2 were shown on RNA-seq. Illumina sequence analysis of the resistant biotypes at CT domain produced 27 single nucleotide variations (SNVs). Out of 27 SNVs, only one non- synonymous mutation, Ile to Leu was found at 1781 position. The amino acid substitution was bolded and underlined, and silent codons were underlined. | | | | |