**Linking metabolites in eight bioactive forage species to their *in vitro* methane reduction potential across several cultivars and harvests**

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**Supplementary Table S1.** Gas production from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in gas production from the cultivar when compared with lucerne, Red\_PEG refers to reduction in gas production from the cultivar in the absence of polyethylene glycol (PEG, a tannin binding agent) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | | | | | **Harvest 2** | | | |
| **-PEG**  **(ml/200mg DM)** | **+PEG**  **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG**  **(ml/200mg DM)** | | **+PEG**  **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 47.93 | 48.12 | -6 | 0 | 44.30 | | 44.07 | 2 | 1 |
| Chicory | Spadona | 54.42 | 53.40 | -20 | -2 | 51.35 | | 51.65 | -15 | -1 |
| Sulla | Grimaldi | 31.27 | 42.11 | 31 | 26 | 45.20 | | 32.48 | 28 | 28 |
| Sulla | Sudda | 26.26 | 46.75 | 42 | 44 | 40.78 | | 25.62 | 43 | 37 |
| Birdsfoot trefoil | Bull | 45.25 | 47.67 | 0 | 5 | 51.54 | | 50.10 | -11 | 3 |
| Birdsfoot trefoil | Lotar | 45.47 | 49.26 | -1 | 8 | 49.97 | | 47.73 | -6 | 4 |
| Birdsfoot trefoil | Rocco | 43.40 | 45.50 | 4 | 5 | 47.93 | | 45.05 | 0 | 6 |
| Big trefoil | Lot 29 | 27.54 | 43.14 | 39 | 36 | 43.60 | | 35.60 | 21 | 18 |
| Big trefoil | Wild type | 31.27 | 41.63 | 31 | 25 | 46.08 | | 42.38 | 6 | 8 |
| Lucerne | Galaxy | 45.17 | 48.10 | 0 | 6 | 47.35 | | 44.78 | 1 | 5 |
| Sainfoin | CPI 63750 | 42.81 | 49.29 | 5 | 13 | 42.33 | | 40.83 | 9 | 4 |
| Sainfoin | Esky | 38.74 | 48.69 | 14 | 20 | 42.77 | | 39.56 | 12 | 7 |
| Sainfoin | Visnovsky | 36.89 | 46.82 | 18 | 21 | 44.75 | | 37.63 | 16 | 16 |
| Plantain | PLA60 | 45.44 | 46.12 | -1 | 1 | 46.23 | | 48.07 | -7 | -4 |
| Plantain | Svatojansky | 32.46 | 33.33 | 28 | 3 | 51.98 | | 51.55 | -14 | 1 |
| Salad burnet | PI 308861 | 38.48 | 46.90 | 15 | 18 | 39.65 | | 27.97 | 38 | 29 |
| Salad burnet | Sang 10 | 44.28 | 49.98 | 2 | 11 | 39.75 | | 32.60 | 28 | 18 |

**Supplementary Table S2**. Methane production from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in methane production from the cultivar when compared with lucerne, Red\_PEG refers to reduction in methane production from the cultivar in the absence of polyethylene glycol (PEG, a tannin binding agent) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | | | | | **Harvest 2** | | | |
| **-PEG**  **(ml/200mg DM)** | **+PEG**  **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG**  **(ml/200mg DM)** | | **PEG**  **(ml/200mg DM)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 9.51 | 9.44 | 12 | -1 | 9.05 | | 8.98 | 16 | -1 |
| Chicory | Spadona | 10.51 | 10.41 | 3 | -1 | 10.67 | | 10.72 | 1 | 0 |
| Sulla | Grimaldi | 7.28 | 9.56 | 33 | 24 | 7.66 | | 10.47 | 29 | 27 |
| Sulla | Sudda | 5.62 | 10.08 | 48 | 44 | 5.85 | | 9.25 | 46 | 37 |
| Birdsfoot trefoil | Bull | 10.91 | 11.17 | -1 | 2 | 11.75 | | 11.34 | -9 | -4 |
| Birdsfoot trefoil | Lotar | 11.02 | 11.53 | -2 | 4 | 12.20 | | 12.40 | -13 | 2 |
| Birdsfoot trefoil | Rocco | 9.85 | 10.48 | 9 | 6 | 11.52 | | 11.97 | -6 | 4 |
| Big trefoil | Lot 29 | 6.79 | 9.86 | 37 | 31 | 8.57 | | 9.98 | 21 | 14 |
| Big trefoil | Wild type | 7.34 | 9.11 | 32 | 19 | 10.04 | | 10.72 | 7 | 6 |
| Lucerne | Galaxy | 10.80 | 10.86 | 0 | 1 | 10.84 | | 11.05 | 0 | 2 |
| Sainfoin | CPI 63750 | 9.44 | 10.47 | 13 | 10 | 9.30 | | 9.88 | 14 | 6 |
| Sainfoin | Esky | 8.55 | 10.64 | 21 | 20 | 9.38 | | 10.23 | 13 | 8 |
| Sainfoin | Visnovsky | 8.72 | 10.26 | 19 | 15 | 8.67 | | 10.17 | 20 | 15 |
| Plantain | PLA60 | 8.67 | 8.93 | 20 | 3 | 9.93 | | 9.33 | 8 | -6 |
| Plantain | Svatojansky | 5.59 | 5.73 | 48 | 3 | 10.47 | | 10.38 | 3 | -1 |
| Salad burnet | PI 308861 | 7.93 | 9.92 | 27 | 20 | 7.05 | | 8.98 | 35 | 22 |
| Salad burnet | Sang 10 | 9.12 | 10.67 | 16 | 15 | 7.73 | | 8.87 | 29 | 13 |

**Supplementary Table S3**. Methane percentage in total gas (MP) from different species and cultivars across the two harvests with and without PEG treatments and the reduction potential of the tested cultivars with respect to control. Red\_MS refers to the reduction in MP from the cultivar when compared with lucerne, Red\_PEG refers to reduction in MP from the cultivar in the absence of polyethylene glycol (-PEG) compared to the treatment with the addition of PEG (+PEG)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Cultivar** | **Harvest 1** | | | | | **Harvest 2** | | | |
| **-PEG**  **(%)** | **+PEG**  **(%)** | **Red\_MS (%)** | **Red\_PEG (%)** | **-PEG**  **(%)** | | **+PEG**  **(%)** | **Red\_MS (%)** | **Red\_PEG (%)** |
| Chicory | Plumato | 19.88 | 19.64 | 17 | -1 | 20.52 | | 20.33 | 15 | -1 |
| Chicory | Spadona | 19.33 | 19.49 | 19 | 1 | 20.72 | | 20.87 | 14 | 1 |
| Sulla | Grimaldi | 23.29 | 22.76 | 3 | -2 | 23.64 | | 23.17 | 2 | -2 |
| Sulla | Sudda | 21.55 | 21.59 | 10 | 0 | 22.90 | | 22.73 | 5 | -1 |
| Birdsfoot trefoil | Bull | 24.16 | 23.47 | -1 | -3 | 23.47 | | 22.08 | 3 | -6 |
| Birdsfoot trefoil | Lotar | 24.46 | 23.45 | -2 | -4 | 25.57 | | 24.78 | -6 | -3 |
| Birdsfoot trefoil | Rocco | 22.82 | 23.21 | 5 | 2 | 25.62 | | 24.95 | -6 | -3 |
| Big trefoil | Lot 29 | 24.98 | 23.07 | -4 | -8 | 24.03 | | 22.88 | 0 | -5 |
| Big trefoil | Wild type | 23.71 | 22.03 | 1 | -8 | 23.66 | | 23.28 | 2 | -2 |
| Lucerne | Galaxy | 23.91 | 22.59 | 0 | -6 | 24.20 | | 23.35 | 0 | -3 |
| Sainfoin | CPI 63750 | 22.13 | 21.32 | 7 | -4 | 22.82 | | 23.40 | 6 | 2 |
| Sainfoin | Esky | 22.09 | 21.90 | 8 | -1 | 23.73 | | 23.93 | 2 | 1 |
| Sainfoin | Visnovsky | 23.72 | 21.95 | 1 | -8 | 23.10 | | 22.80 | 4 | -1 |
| Plantain | PLA60 | 19.07 | 19.36 | 20 | 1 | 20.67 | | 20.35 | 14 | -2 |
| Plantain | Svatojansky | 17.19 | 17.20 | 28 | 0 | 20.35 | | 19.98 | 16 | -2 |
| Salad burnet | PI 308861 | 20.70 | 21.20 | 13 | 2 | 25.29 | | 22.67 | -5 | -12 |
| Salad burnet | Sang 10 | 20.61 | 21.45 | 14 | 4 | 23.75 | | 22.30 | 2 | -7 |