Investigating Association Between Leafy Greens Metabolome and Surface Metabolome and *Salmonella* Growth Under Drought Using Electrospray Ionization Mass-Spectrometry (ESI-MS) Analysis

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INTRODUCTION

- *Salmonella enterica* can survive epiphytically, utilizing compounds released and exuded to plant surfaces. Factors impacting plant physiological profiles may play a role in plant-*Salmonella* association.
- When plants experience drought stress in hot weather, during dry spells and in between irrigation, their phytochemical profiles may shift to help tolerate the stress (M. Faroog et al., 2009).
- Phytochemical profiles also change over the course of plant development (Rajabbeigi et al., 2013 & Myung et al., 2010).

HYPOTHESIS

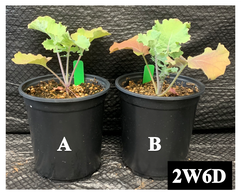
- Mature kale plants, and plants under drought stress accumulate more secondary metabolites that impede the growth of *Salmonella enterica*.

EXPERIMENTAL PLANS

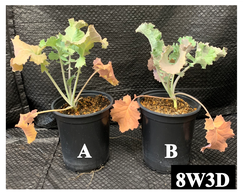
Brassica napus ('Improved dwarf Siberian Kale')

Separate 2 or 8 weeks old kale plants randomly into two treatments:
A: regular watering (control)
B: watering withheld for 3 or 6 days (drought)

Juvenile kale (20 days old)



Mature kale (59 days old)



S. Newport Epiphytic growth → Retrieval & Enumeration on TSA amended with rifampicin

S. Newport growth in leaf wash → Retrieval after 24 hours & enumeration on plates

Biochemical measurements:
- whole plant

→ Absorbance reading at specific wavelength

Phytochemical profiling:
- whole plant
- leaf wash

→ ESI-MS profiles under negative and positive modes

Data analysis

RESEARCH RESULTS

- *Salmonella* epiphytic growth on kale was impacted by plant age and drought stress.
  - ➢ Age effects:
    Plant age negatively affected epiphytic *S.* Newport association with kale.
    – Control: the retrieval of *S.* Newport from juvenile kale surface was higher than mature plant surface ($p < 0.05$).
    – Drought-exposed: the retrieval of *S.* Newport was significantly reduced on mature kale surface compared to juvenile kale surface ($p < 0.05$).
  - ➢ Drought effects:
    Drought-exposed kale plants supported lower levels of *S.* Newport than regularly watered plants.
    – Juvenile: the retrieval of *S.* Newport from control surface was higher than drought-exposed plant surface ($p < 0.05$).
- *Salmonella* growth in leaf wash after 24 hours varied by drought stress and plant age.
  - ➢ Age effects:
    – Control: the growth of *S.* Newport in juvenile kale wash was higher than in mature plant leaf wash ($p < 0.05$).
    – Drought-exposed: the growth of *S.* Newport in leaf wash of juvenile plants was significantly higher than in mature plant leaf washes ($p < 0.05$).
  - ➢ Drought effects:
    – Juvenile: the growth of *S.* Newport in control leaf wash was higher than in washes collected from drought-exposed plant ($p < 0.05$).

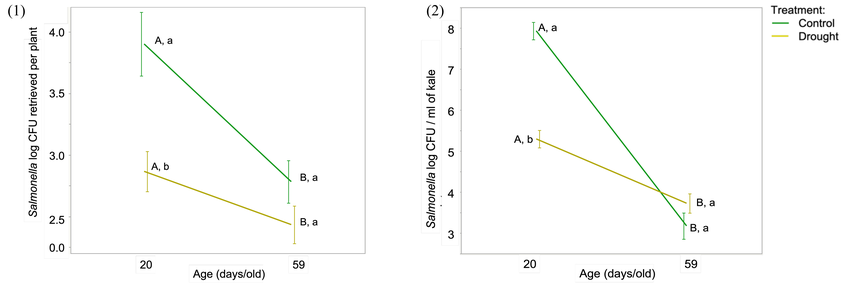


Figure 1. Levels of *Salmonella* Newport on (1) leaf surfaces of regularly-watered and drought-exposed juvenile (20 days old) and mature (59 days old) kale plants and (2) in plant leaf washes. Capital letters denote differences due to age under the same watering regime (ANOVA and Tukey HSD; $p < 0.05$). Small letters indicate differences by treatment within same age group (Student's t-test, $p < 0.05$). Capital letters denote differences due to age under the same watering regime (ANOVA and Tukey HSD; $p < 0.05$). Small letters indicate differences by treatment within same age group (Student's t-test, $p < 0.05$) .

- Biochemical analyses of kale leaves indicated differences due to plant age and drought stress.
  - ➢ Age effects:
    – Control: mature plants showed higher antioxidant capacity, accumulation of total phenolics and flavonoids ($p < 0.05$).
    – Drought-exposed: mature plants showed higher antioxidant capacity and lower levels of glucosinolates ($p < 0.05$).
  - ➢ Drought effects:
    – Juvenile: drought-exposed plants showed higher antioxidant capacity, accumulation of total phenolics, flavonoids and glucosinolates ($p < 0.05$).

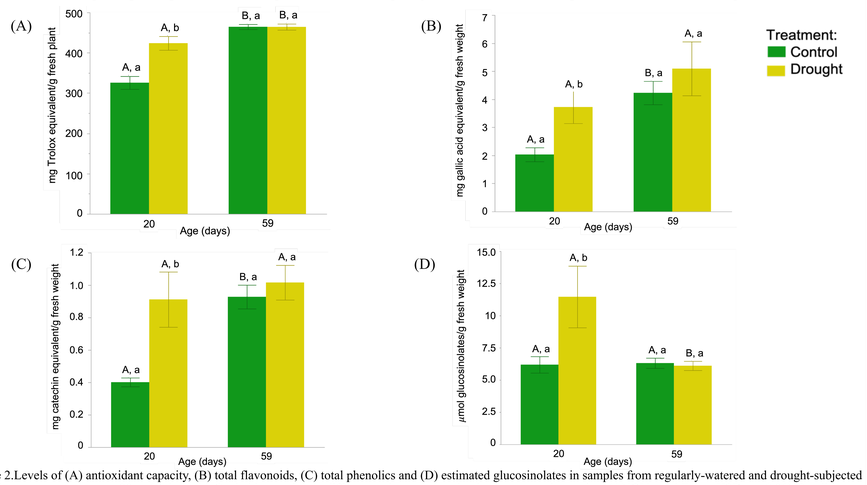


Figure 2.Levels of (A) antioxidant capacity, (B) total flavonoids, (C) total phenolics and (D) estimated glucosinolates in samples from regularly-watered and drought-subjected kale plants at two age stages: 20 or 59 days old. Capital letters denote differences due to age under the same watering regime (ANOVA and Tukey HSD; $p < 0.05$). Small letters indicate differences by treatment within same age group (Student's t-test, $p < 0.05$) .

- Phytochemical profiling of kale leaf tissue extracts and leaf surface compounds using ESI-MS revealed marked differences between leaf tissue and leaf surface metabolomes.

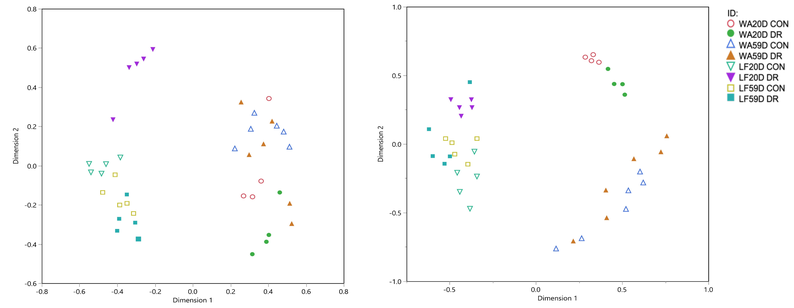


Figure 3. Multidimensional scaling (MDS) of both kale leaf and leaf wash samples from ESI-MS profiles under (A) positive mode and (B) negative mode.

- Phytochemical profiling of leaf tissue extracts, and leaf washes revealed age-related and drought stress-induced shifts.
  - ❑ Tissue extracts:
    ❖Age effects: profiles retrieved from mature kale plants and juvenile plants diverged under both negative and positive mode ($p < 0.05$).
    ❖Drought effects: profiles retrieved from regularly watered juvenile kale plants clustered differently with drought-exposed juvenile kale plants under both negative and positive mode ($p < 0.05$).
  - ❑ Leaf washes:
    ❖Age effects: profiles retrieved from mature kale plants and juvenile plants diverged under negative mode ($p < 0.05$).
    ❖Drought effects: profiles retrieved from regularly watered juvenile kale plants clustered differently with drought-exposed juvenile kale plants under both negative and positive mode ($p < 0.05$).

CONCLUSION

- *Salmonella* association was most successful on juvenile plants and decreased as plants matured.
- *Salmonella* interaction was impaired on kale suffering water stress, regardless of plant developmental stage.
- Kale surface phytochemicals collected in leaf washes were capable of supporting the growth of *Salmonella*.
- Higher accumulation of total flavonoids and phenolics may restrict *Salmonella* colonization. However, higher glucosinolates did not cause *Salmonella* restriction.
- Increases in total phenolic and flavonoids contents might partially explain the age-dependent metabolomics shifts and drought-driven metabolome changes in juvenile, but not mature kale.
- Kale phytochemical profiles changed with plant ontogeny. At juvenile stage, but not the mature stage, phytochemical profiles shifted markedly in response to mild drought treatment.
- Drought in juvenile plants and plant age impacted the exometabolome profiles of kale leaves.

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