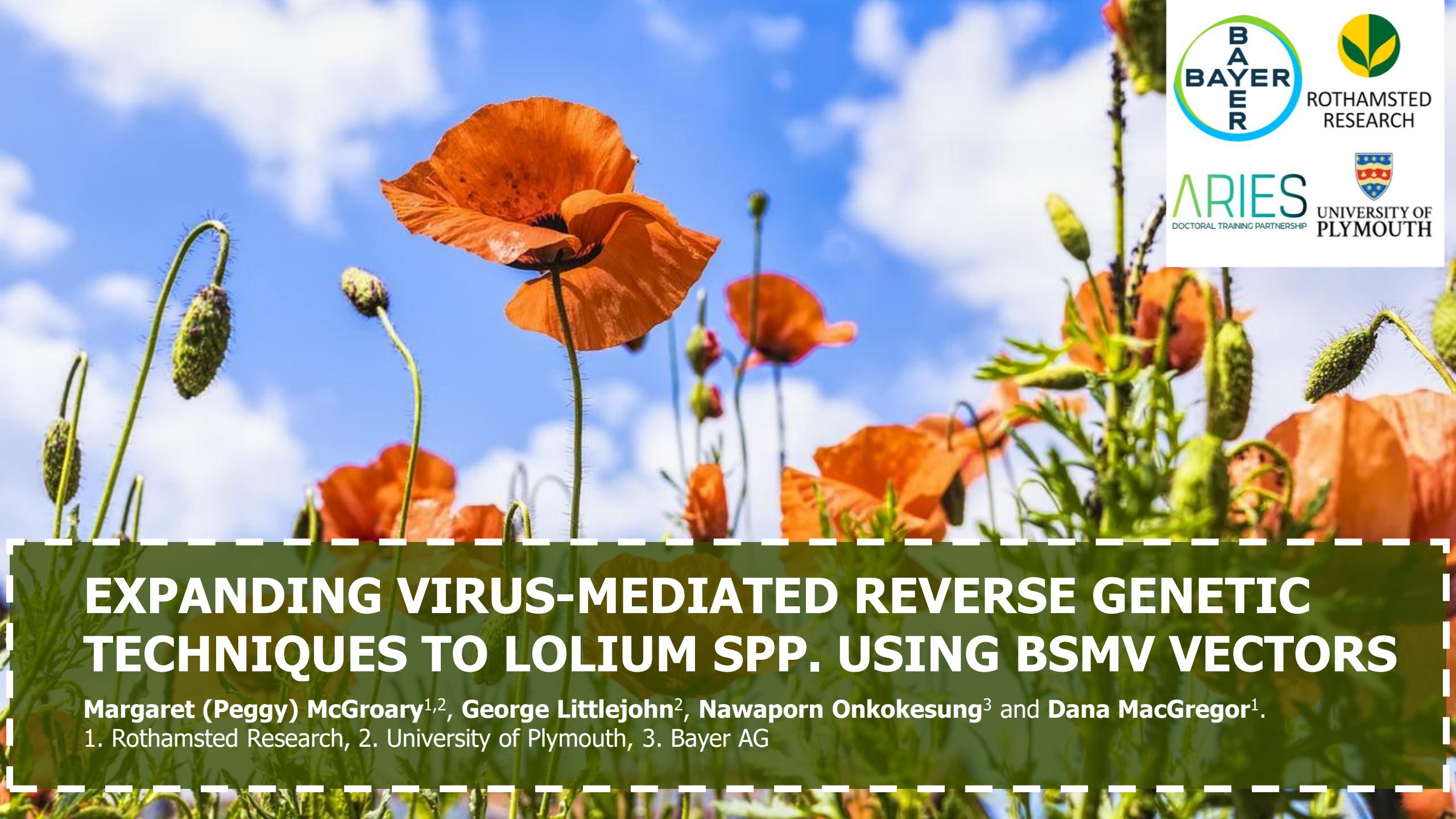

# EXPANDING VIRUS-MEDIATED REVERSE GENETIC TECHNIQUES TO LOLIUM SPP. USING BSMV VECTORS

**Margaret (Peggy) McGroary**[1,2], **George Littlejohn**[2], **Nawaporn Onkokesung**[3] and **Dana MacGregor**[1].
1. Rothamsted Research, 2. University of Plymouth, 3. Bayer AG


Lolium spp.
A global weed problem





# GLOBAL MAP OF MOST IMPORTANT WEEDS AFFECTING CROP PRODUCTION

HERBICIDE RESISTANCE ACTION COMMITTEE



**CEREALS, CANOLA, PULSES**

- *Amaranthus retroflexus*
- *Avena fatua*
- *Cirsium arvensis*
- *Galium spurium*
- *Bassia scoparia*
- *Setaria viridis*
- *Stellaria media*

**CEREALS, CORN, OILSEED RAPE, POTATO, SUGARBEET, PERENNIAL CROPS**

- *Alopecurus myosuroides*
- *Amaranthus* spp.
- *Apera spica-venti*
- *Bromus sterilis*
- *Centaurea cyanus*
- *Erigeron canadensis*
- *Echinochloa* spp.
- *Galium aparine*
- *Lolium* spp.
- *Matricaria* spp.
- *Papaver rhoeas*
- *Stellaria media*

**RICE**

- *Cyperus* spp.
- *Echinochloa crus-galli & oryzicola*
- *Eleusine indica*
- *Fimbristylis miliacea*
- *Ischaemum rugosum*
- *Leptochloa chinensis & panicea*
- *Monochoria vaginalis*
- *Sagittaria trifolia*
- *Scirpus juncoides*
- *Spenochlea zeylanica*

**WHEAT**

- *Chenopodium album*
- *Phalaris minor*
- *Rumex dentatus*

**PLANTATION**

- *Eleusine indica*

**RICE, WHEAT**

- *Alopecurus aequalis*
- *Echinochloa* spp.
- *Eleusine indica*
- *Monochoria* spp.
- *Sagittaria trifolia*
- *Schoenoplectus juncoides*
- *Lindernia* spp.

**RICE**

- *Cyperus* spp.
- *Digitaria sanguinalis.*
- *Echinochloa crus-galli & oryzoides*
- *Leptochloa chinensis*
- *Ludwigia prostrata*
- *Monochoria* spp.
- *Sagittaria trifolia*
- *Scirpus* spp.

**WHEAT**

- *Aegilops tauschii*
- *Alopecurus aequalis, japonicus & myosuroides*
- *Beckmaania syzigachne*
- *Lolium multiflorum*
- *Veronica polita*

**CORN & SOYBEAN**

- *Amaranthus retroflexus*
- *Chenopodium album*
- *Commelina communis*
- *Setaria* spp.

**CORN, SOYBEAN, COTTON**

- *Amaranthus palmeri & tuberculatus*
- *Ambrosia trifida & artemsiifolia*
- *Chenopodium album*
- *Erigeron* spp.
- *Ipomoea* spp.
- *Bassia scoparia*
- *Sorghum halepense*

**SOYBEAN, CORN, COTTON & CEREALS**

- *Amaranthus* spp.
- *Bidens* spp.
- *Erigeron* spp.
- *Digitaria insularis*
- *Echinochloa* spp.
- *Eleusine indica*
- *Euphorbia heterophylla*
- *Lolium multiflorum*
- *Spermacoce spp.*

**SOYBEAN, CORN & CEREALS**

- *Amaranthus hybridus*
- *Erigeron bonariensis & canadiensis*
- *Echinochloa* spp.
- *Eleusine indica*
- *Lolium multiflorum*
- *Sorghum halepense*

**CORN, SUGARCANE, PERENNIAL CROPS, SOYBEAN**

- *Amaranthus palmeri & hybridus*
- *Bromus diandrus*
- *Erigeron bonariensis & canadensis*
- *Cynodon dactylon*
- *Ipomoea purpurea*
- *Lolium multiflorum*
- *Rottboelia cochinchinensis*
- *Sorghum halepense*

**CEREALS, CANOLA, PULSES**

- *Avena* spp.
- *Bromus* spp.
- *Erigeron* spp.
- *Hordeum leporinum*
- *Lolium rigidum*
- *Raphanus raphanistrum*
- *Sisymbrium orientale*
- *Sonchus serriola*

**Sources:**
Regional HRAC (EU, US, ASIA, JP, BR, ARG, ZA, AUS)

* Indian + Pakistan

# GLOBAL MAP OF MOST IMPORTANT WEEDS AFFECTING CROP PRODUCTION

HERBICIDE RESISTANCE ACTION COMMITTEE




**Sources:**
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* Indian + Pakistan



# Virus-Induced Gene Silencing (VIGS)

- Targets the **RNAi Pathway**
- RNA produced by the infecting virus is targeted for **sequence-specific degradation**
- By utilising a fragment of a **gene-of-interest** into a suitable viral vector, matching host RNA will also become targets for degradation
- Results in a **reduced expression** of the target gene

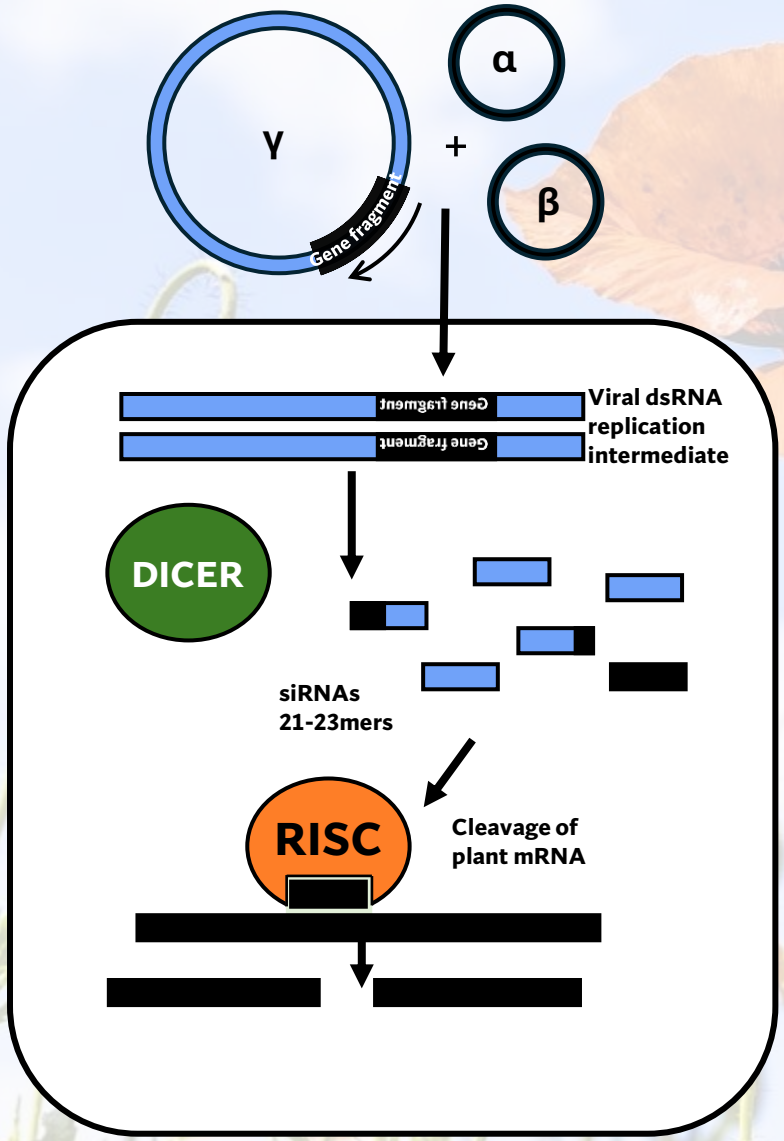



Illustration of VIGS utilising a BSMV vector

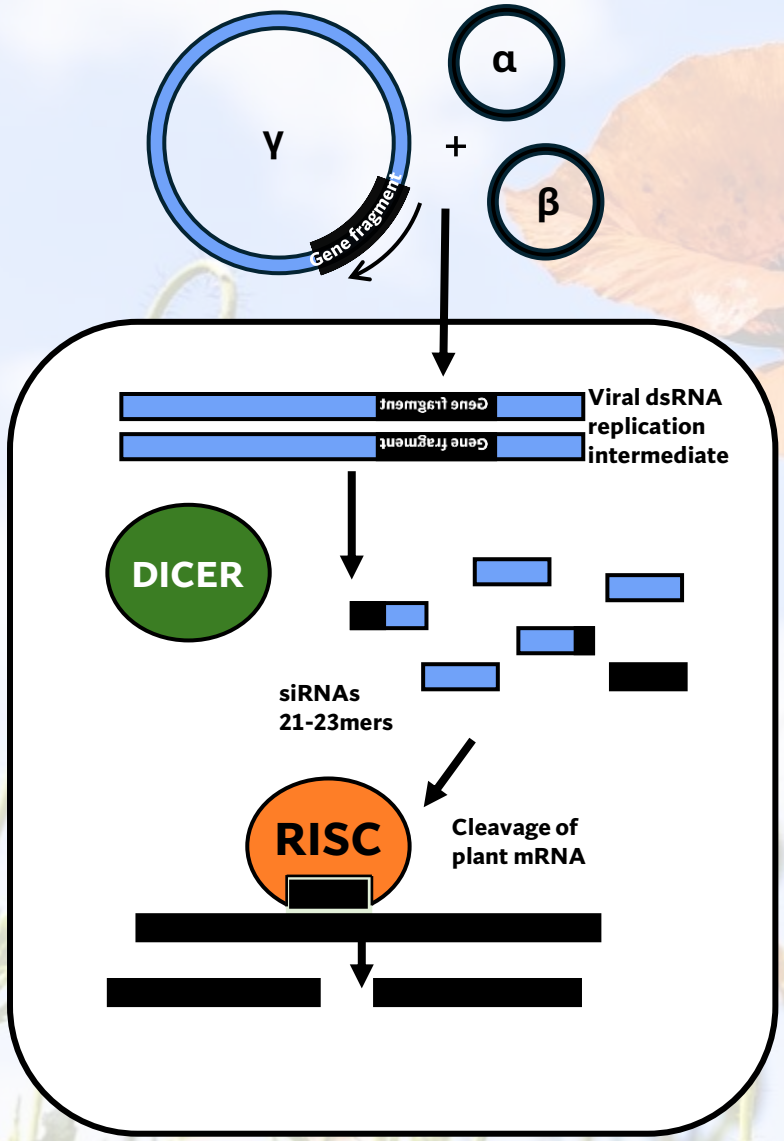
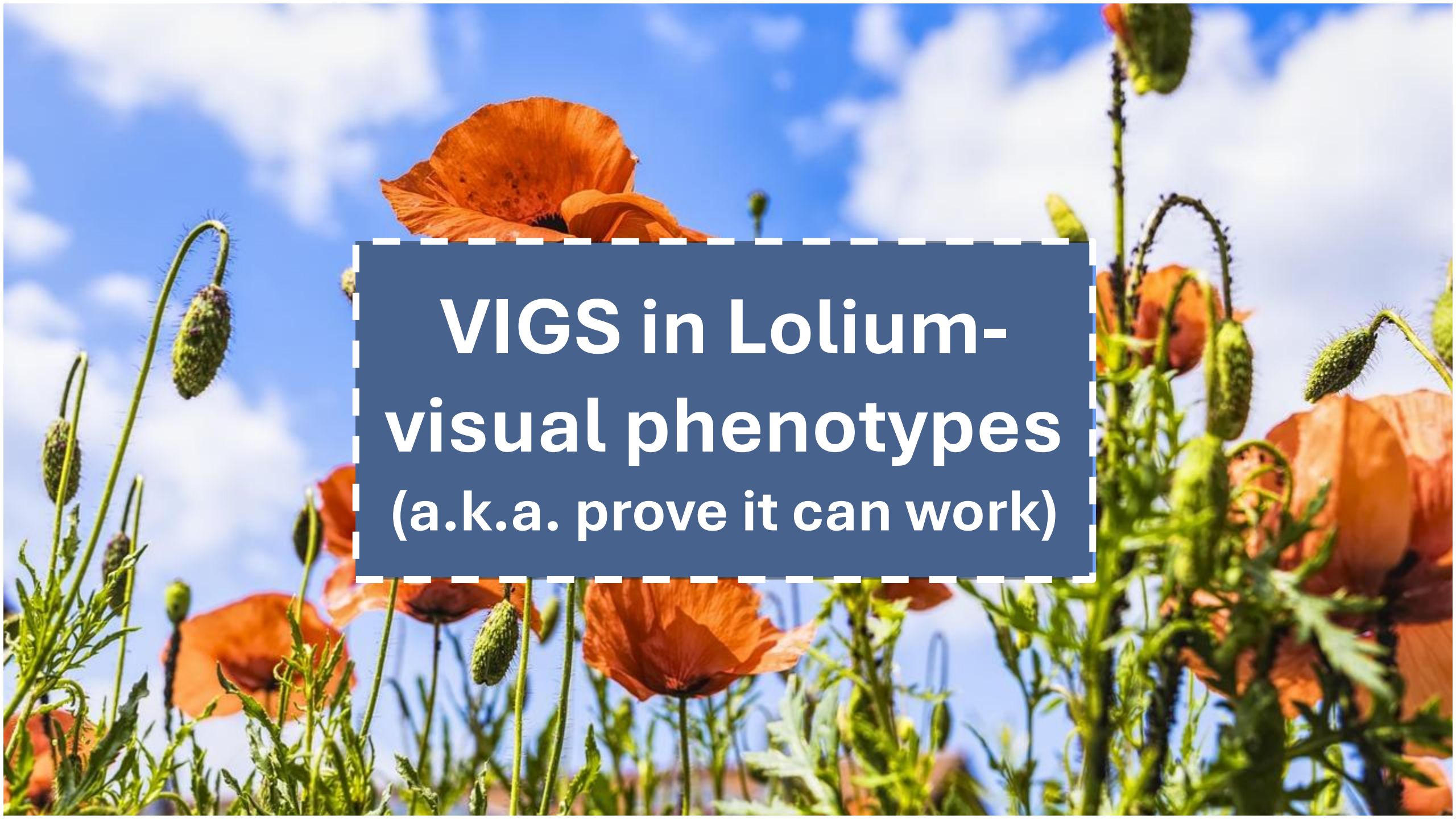



Illustration of VIGS utilising a BSMV vector

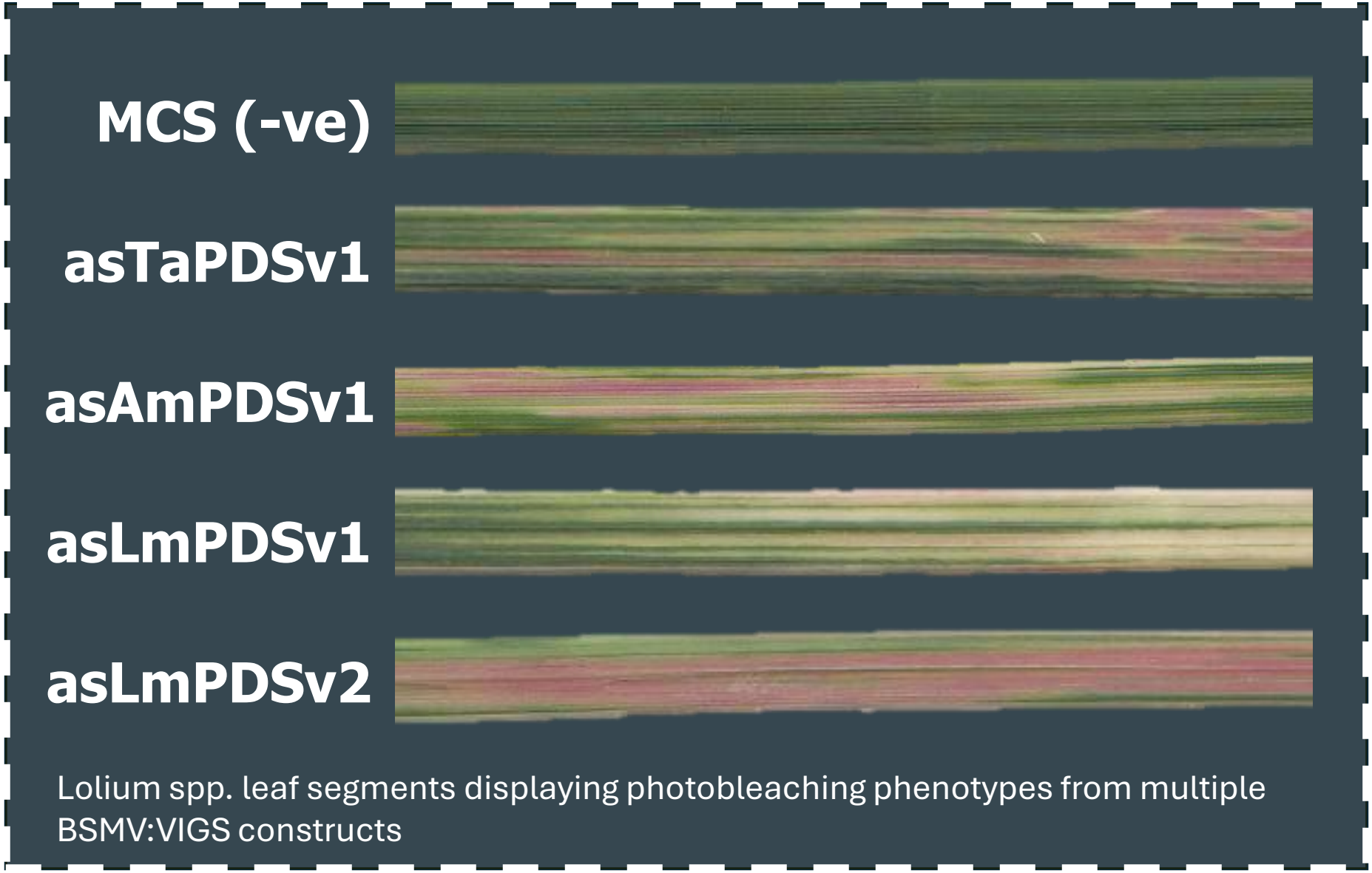
# Advantages and disadvantages

- Suitable for species in which **stable transformation** is not possible (such as *Lolium*)
- Can target genes that would otherwise be **fatal**
- Relatively **high-throughput**
- Limited to **functional validation**
- Some limits on applications

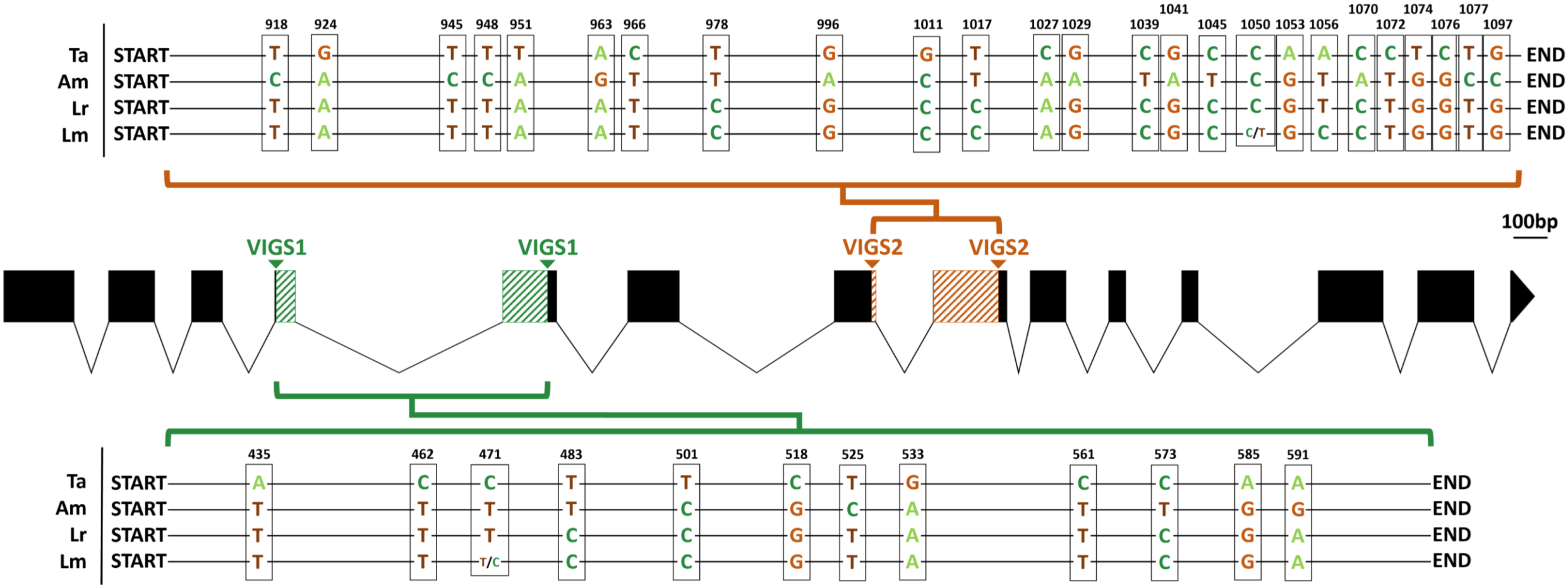

VIGS in Lolium-
visual phenotypes
(a.k.a. prove it can work)




Images of *Lolium multiflorum* CLEV successfully expressing photobleaching phenotype from VIGS constructs BSMV:asAmPDSv1. A) 31 DPI. 10 days after nodes were cut. On day 50 plants were placed in vernalisation. B) 86 DPI. Plants were removed from vernalisation C) 113 DPI. 3 days before phenotype declared absent




Lolium spp. leaf segments displaying photobleaching phenotypes from multiple BSMV:VIGS constructs




PDS VIGS1 and VIGS2 segment alignments for Wheat- *Triticum aestivum* (Ta), Black-grass- *Alopecurus myosurides* (Am), Rigid Ryegrass- *Lolium rigidum* (Lr), Italian Ryegrass- *Lolium multiflorum* (Lm). Nucleotide variations are highlighted and distances between bases are proportional to true distances.

| BSMV: Construct | *Lolium rigidum* | | | *Lolium multiflorum* | | *Lolium* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | KEW-1 | KEW-2 | KEW-3 | CLEV | RIPPLE | FRA-4 | FRA-6 | DEU-21 | DEG-19 |
| **asAmPDSv1** | 0% | 22% | 11% | 11-14% | 0% | 20-45% | ≤9% | 4-6% | 0% |
| **asTaPDSv1** | - | - | - | 14-25% | 0% | 16-45% | ≤20% | ≤30% | 0% |
| **asLmPDSV1** | 0% | 78% | 38% | 35% | - | 68% | 40% | 55% | - |
| **asLmPDSV2** | 0% | 56% | 0% | 10% | - | 53% | 30% | 10% | - |

**Summary of the efficiency (percentage of affected plants) of multiple constructs in Lolium spp. including multiple biotypes over multiple experiments**

| BSMV: Construct | *Lolium rigidum* | | | *Lolium multiflorum* | | *Lolium* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | KEW-1 | KEW-2 | KEW-3 | CLEV | RIPPLE | FRA-4 | FRA-6 | DEU-21 | DEG-19 |
| **asAmPDSv1** | 0% | 22% | 11% | 11-14% | 0% | 20-57% | ≤9% | 4-14% | 0% |
| **asTaPDSv1** | - | - | - | 14-25% | 0% | 17-45% | ≤20% | ≤30% | 0% |
| **asLmPDSV1** | 0% | 78% | 38% | 35% | - | 74-100% | 40% | 70% | - |
| **asLmPDSV2** | 0% | 56% | 0% | 10% | - | 50-63% | 30% | 10% | - |

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| BSMV: Construct | *Lolium rigidum* | | | *Lolium multiflorum* | | *Lolium* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|
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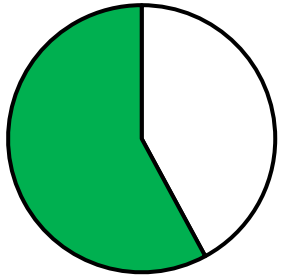
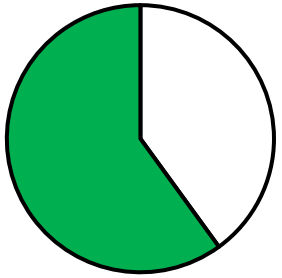
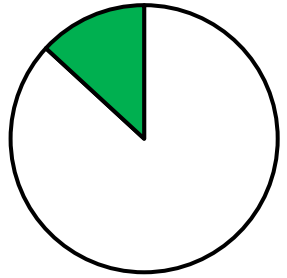
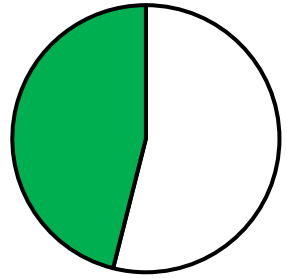
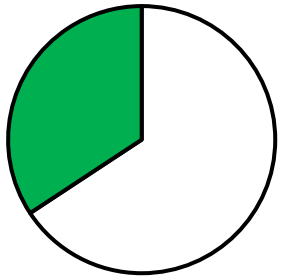
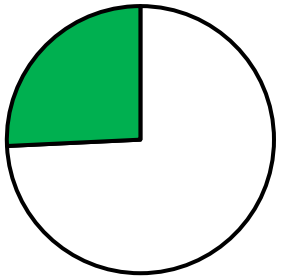
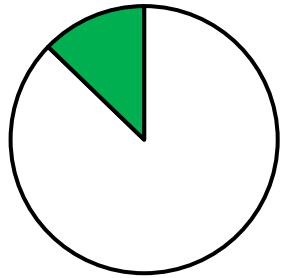
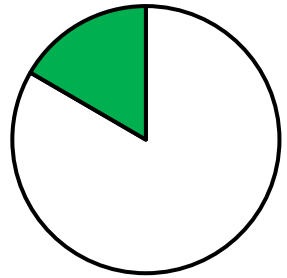
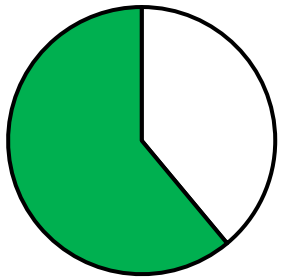
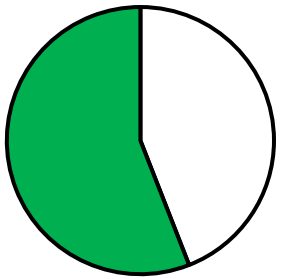
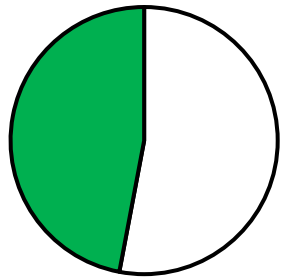
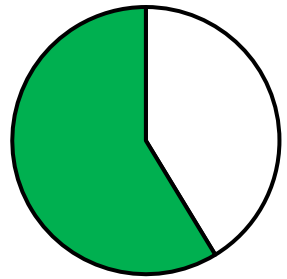
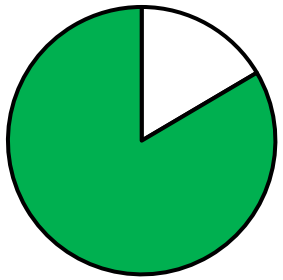
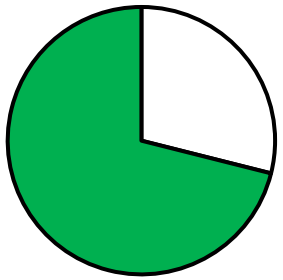
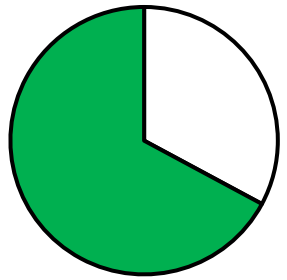
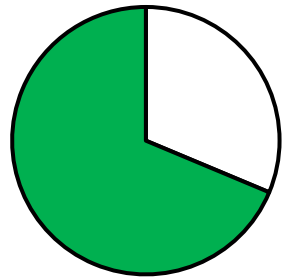
**Summary of the efficiency (percentage of affected plants) of multiple constructs in Lolium spp. including multiple biotypes over multiple experiments**

| | asTaPDSv1 | asAmPDSv1 | asLmPDSv1 | asLmPDSv2 |
|---|---|---|---|---|
|  Percentage of affected plants |  |  |  |  |
|  Percentage of affected tillers |  |  |  |  |
|  Percentage of affected leaves |  |  |  |  |
|  Percentage of affected leaf surface |  |  |  |  |



Percentage of affected plants



Percentage of affected tillers

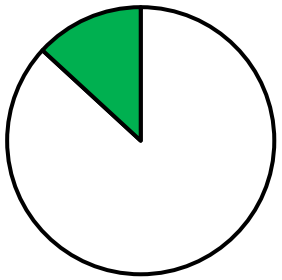


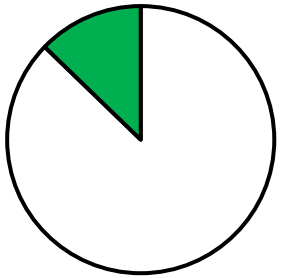
Percentage of affected leaves

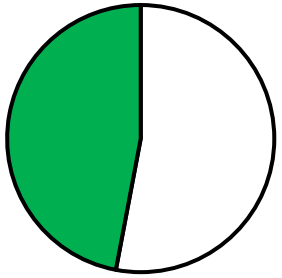


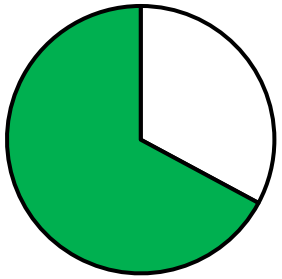
Percentage of affected leaf surface

asLmPDSv1







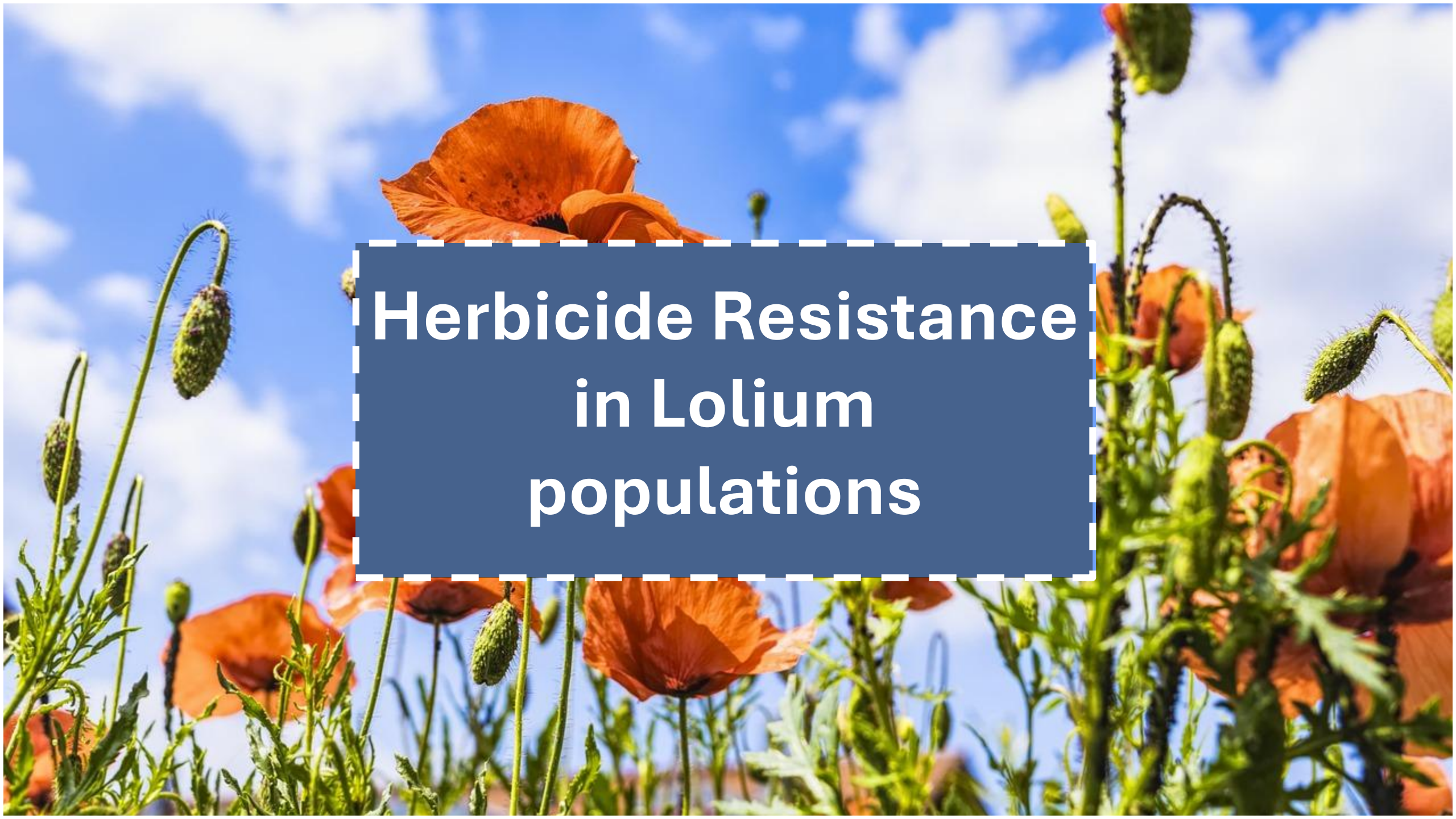


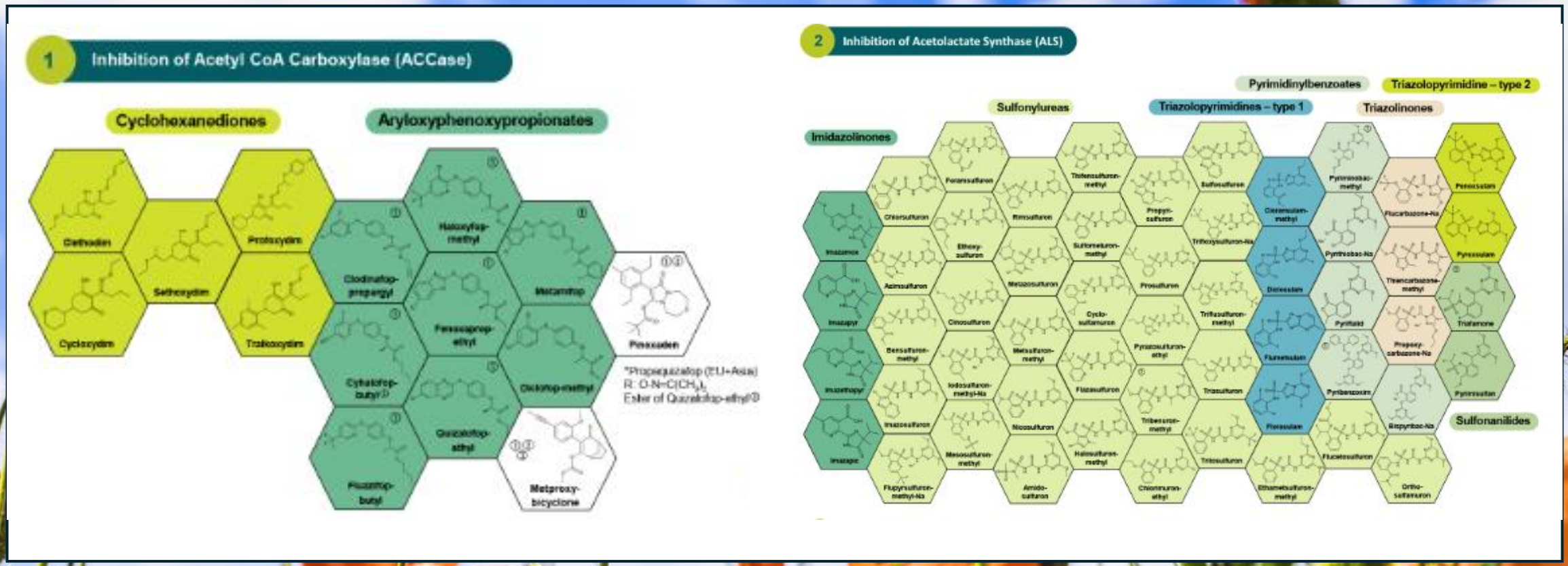
Approximate percentage of affected plant tissue (if inoculation success is unknown)

7.6% (1.8-14%)

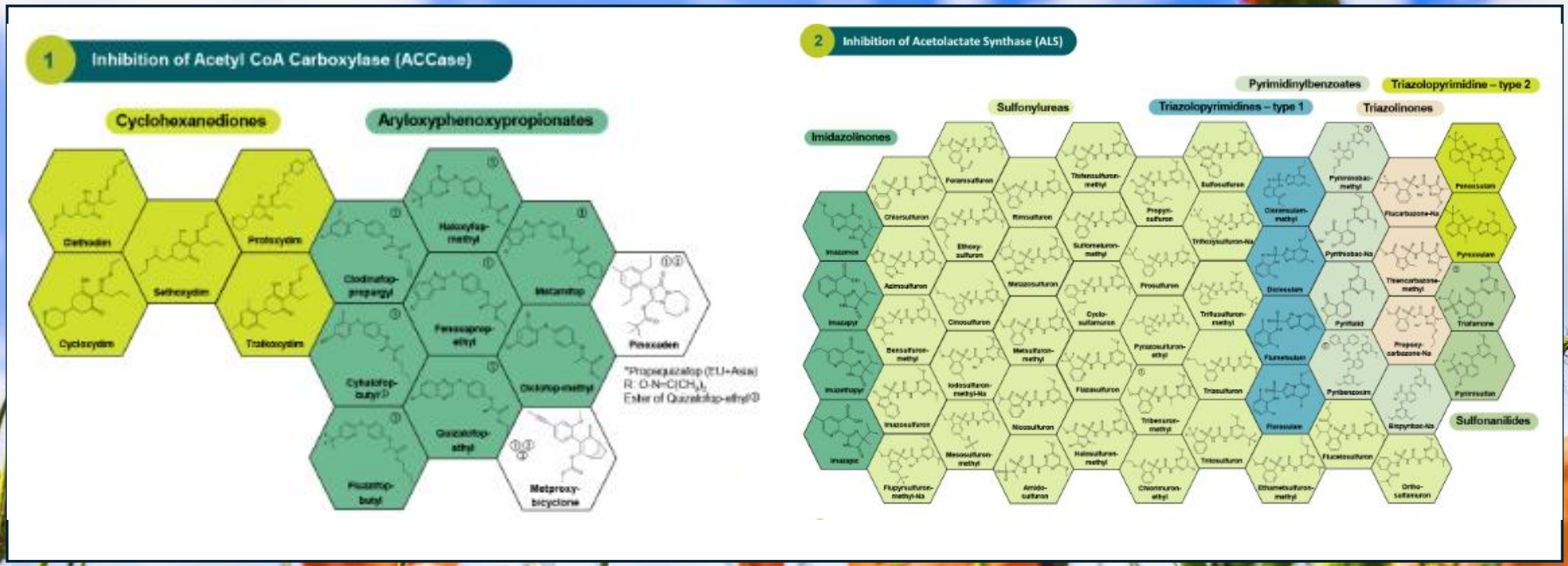
Approximate percentage of affected plant tissue (if inoculation success is known)

14%


Herbicide Resistance in Lolium populations

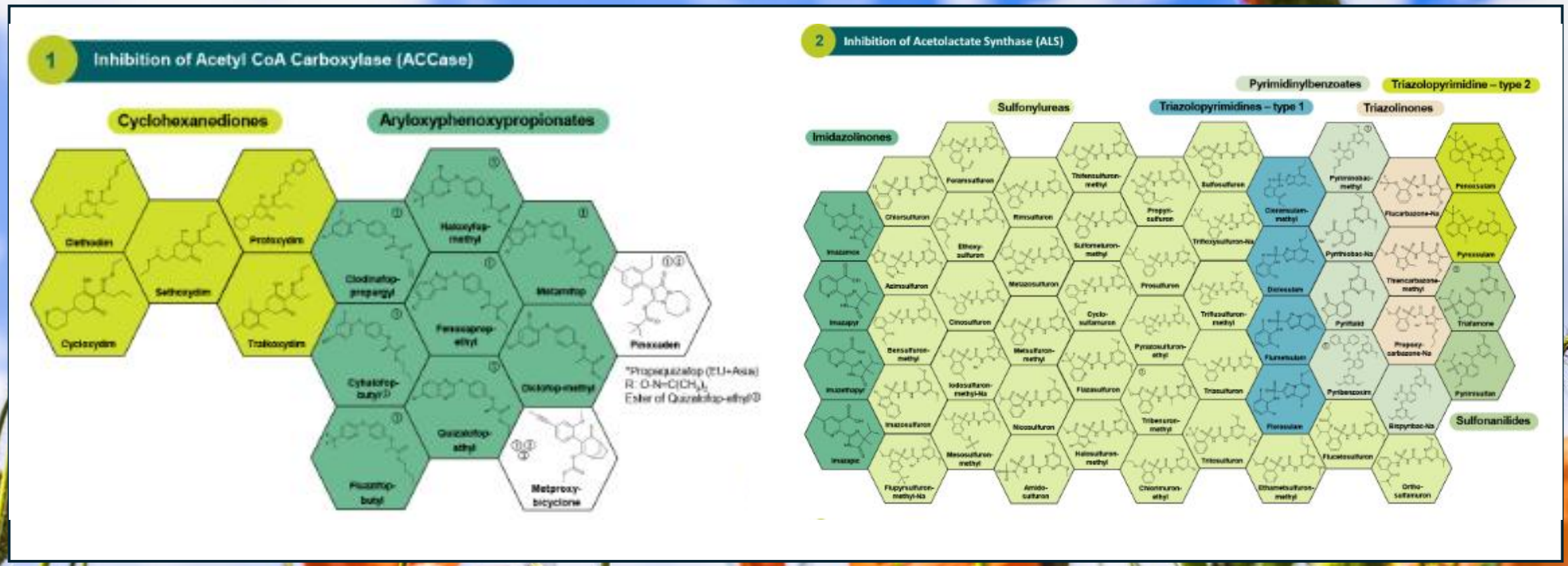



HRAC classes Loliums as **high risk** for developing resistance Class 1 and 2 herbicides




Mutations in **ACCase** cause resistance to group 1 herbicides

Mutations in **ALS** cause resistance to group 2 herbicides




Alternatively, **metabolic resistances** also develop rapidly in *Lolium*, resulting in **multiple herbicide resistance**.

For example, CYPs **detoxify** the herbicide, so its less harmful to the plant

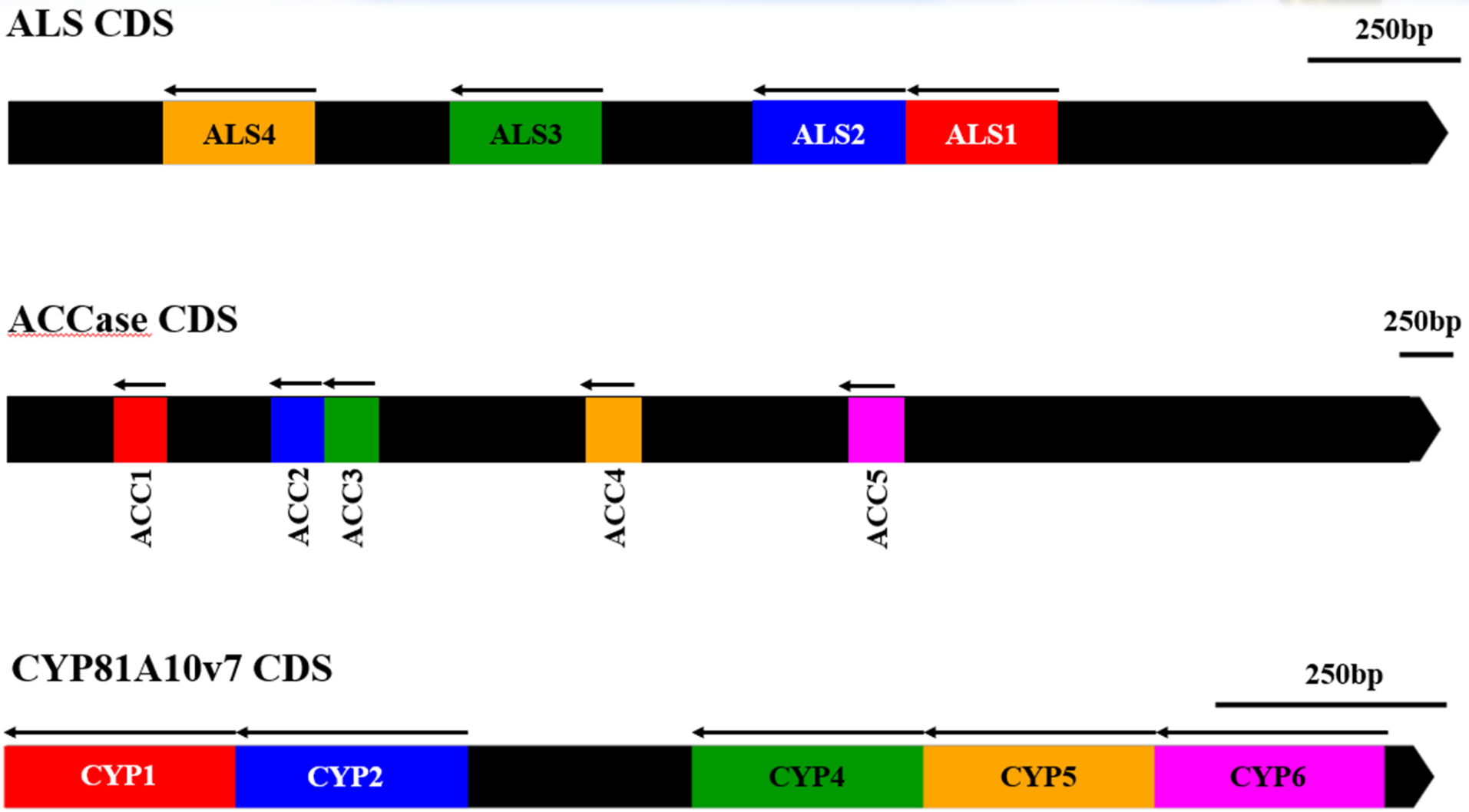


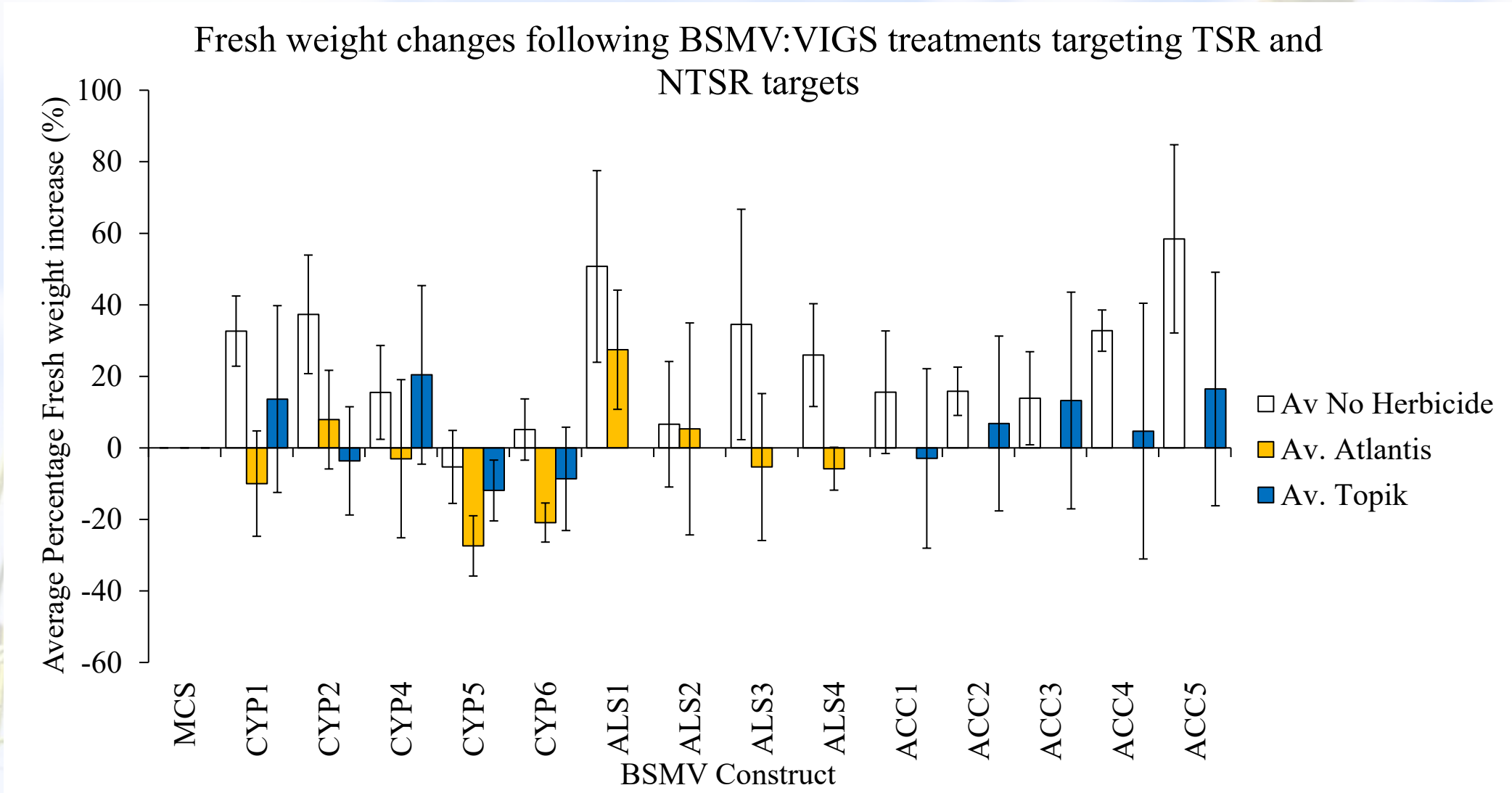
## Targets:

TSR:
ACCase
ALS

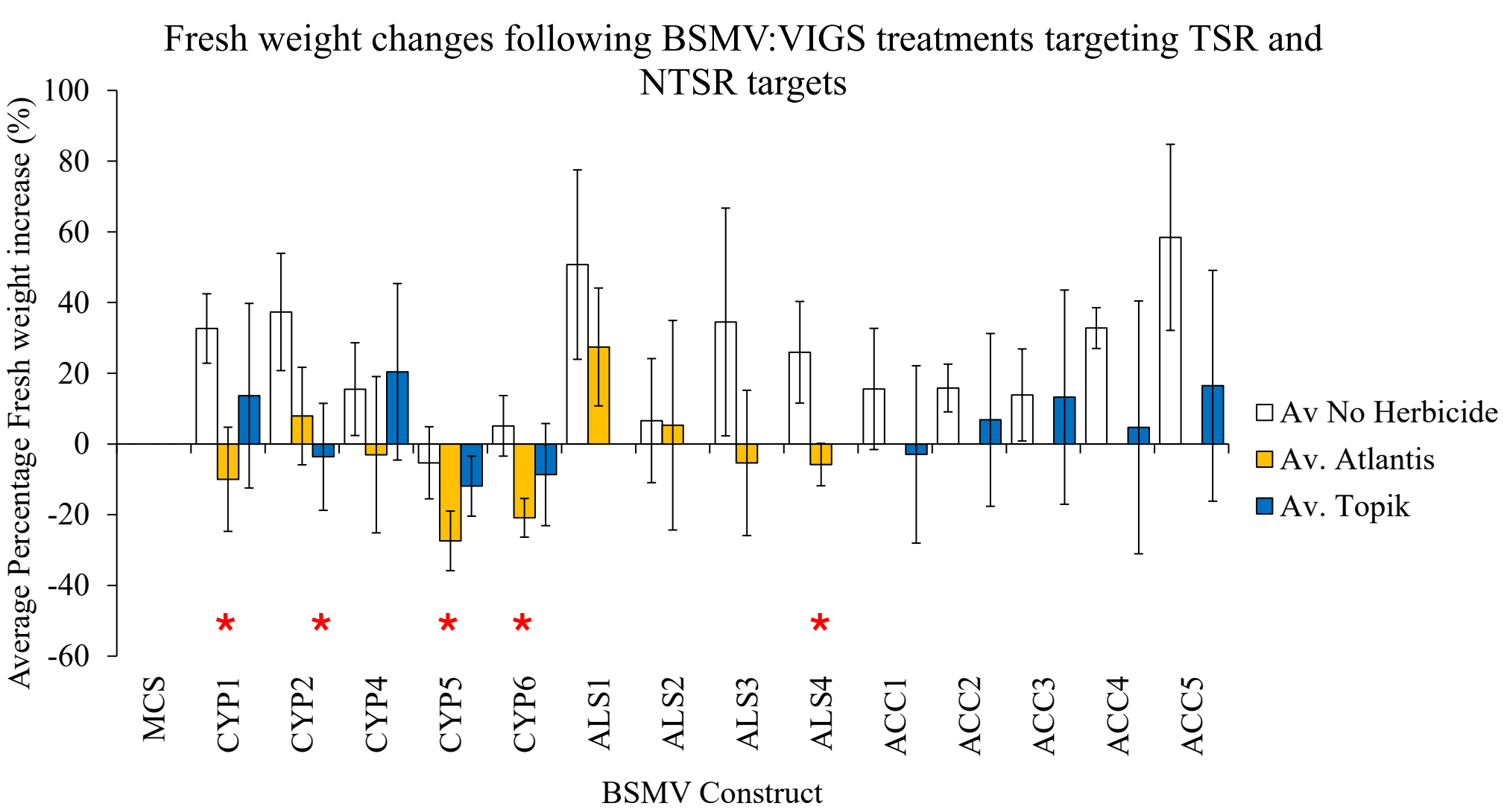
NTSR:
CYP81A10v7 (cytochrome p450)

Will be used to determine if these genes are **sufficient** and **necessary** to cause herbicide resistance phenotypes


ALS CDS
250bp
ALS4
ALS3
ALS2
ALS1
ACCase CDS
250bp
ACC1
ACC2
ACC3
ACC4
ACC5
CYP81A10v7 CDS
250bp
CYP1
CYP2
CYP4
CYP5
CYP6




A bar graph displaying the average change in fresh weights normalised to MCS treatments across multiple experiments. The error bars display the SEM.




A bar graph displaying the average change in fresh weights normalised to MCS treatments across multiple experiments. The error bars display the SEM.



# THANK YOU FOR LISTENING, ANY QUESTIONS?

ROTHAMSTED RESEARCH

UNIVERSITY OF PLYMOUTH

BAYER

ARIES
DOCTORAL TRAINING PARTNERSHIP