

Ecological Applications Topic: **Atrazine**
Rep. E AIQaffas

The problem:

-Weeds growing in maize via a competitive relationship lowering maize yield by 40 %. (Chikoye, 2004).

Proposed solution:

- genetically editing of maize in order to increase its resistance to glyphosate.
- spread GM maize and weeds with glyphosate.

- History (Ren, 2015)

- Translocated genes: glyphosate-insensitive *5-enolpyruvyl shikimate 3-phosphate synthase*
- Gene sources: a REDISGIN AM79 *aroA* from *Escherichia coli* (where the WT sequence was labeled WO2009/059485).

Protein Alignment between the two sequences provided with provided label:

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moqf tteq *****
oi r t y u *****
moqf tteq БСЛАЕКМСВАЛЕЕТОКЛОГНАЕЛИ 442
oi r t y u БСЛАЕКМСВАЛЕЕТОКЛОГНАЕЛИ 442
moqf tteq *****
oi r t y u *****
moqf tteq ТУАТСБГООМСАДАЕЕНЕДСЛЛАБОБАЛЛИГМНДНВНИМЛАСТСАКАЛНИКИАД 450
oi r t y u ТУАТСБГООМСАДАЕЕНЕДСЛЛАБОБАЛЛИГМНДНВНИМЛАСТСАКАЛНИКИАД 450
moqf tteq *****
oi r t y u *****
moqf tteq АЛКИЕВЛГЕАЛСЪЛГКОСЪЕАДЖКЪНБДОВГЛЮУГЪВЪУДЪИВАЛИМЪИЪУВЕДЪ 360
oi r t y u АЛКИЕВЛГЕАЛСЪЛГКОСЪЕАДЖКЪНБДОВГЛЮУГЪВЪУДЪИВАЛИМЪИЪУВЕДЪ 360
moqf tteq *****
oi r t y u *****
moqf tteq АЛЬЛОСНДЛИГЕВДЪУСЪЛГЪУУГЛОСЛОАКИАСНЪЛОЪУВЪИДАГОМЪСЪ 300
oi r t y u АЛЬЛОСНДЛИГЕВДЪУСЪЛГЪУУГЛОСЛОАКИАСНЪЛОЪУВЪИДАГОМЪСЪ 300
moqf tteq *****
oi r t y u *****
moqf tteq СНАБЪОЛГРЪГТИВЪАУВЕВЪАГЕАИЪОЪЕЛЪИЪИЛОУМЪЕОУКЪАНИЪОУКЪЪ 340
oi r t y u СНАБЪОЛГРЪГТИВЪАУВЕВЪАГЕАИЪОЪЕЛЪИЪИЛОУМЪЕОУКЪАНИЪОУКЪЪ 340
moqf tteq *****
oi r t y u *****
moqf tteq АУООСЕНАДЪАЪОУЪЕНЪКЪАДЪУГЛОСЪКЪЕАГЪЕНЪСЪГЪАКЪУГЪСОУАНАЪ 180
oi r t y u АУООСЕНАДЪАЪОУЪЕНЪКЪАДЪУГЛОСЪКЪЕАГЪЕНЪСЪГЪАКЪУГЪСОУАНАЪ 180
moqf tteq *****
oi r t y u *****
moqf tteq ГКБДЪАМСИДЪГЪКЪГКИЕАВЕЕЛАЛИНЪСОСЪМЪЛОУВЕГЪИУУГЪЛИВЪЪУГЪ 150
oi r t y u ГКБДЪАМСИДЪГЪКЪГКИЕАВЕЕЛАЛИНЪСОСЪМЪЛОУВЕГЪИУУГЪЛИВЪЪУГЪ 150
moqf tteq *****
oi r t y u *****
moqf tteq ИНЪАЪЕЪЪМЪКЪАЕНЪУАЪЪЕНЪКЪИЪСЪЛЪЪСЪКЪАЛИНЪУТИУУГЪЕОЪЕЛЪКЪИ 00
oi r t y u ИНЪАЪЕЪЪМЪКЪАЕНЪУАЪЪЕНЪКЪИЪСЪЛЪЪСЪКЪАЛИНЪУТИУУГЪЕОЪЕЛЪКЪИ 00

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DNA alignment between the two:

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origina atgtcacattctaccttagtccccatggtccaaggctactgagaccatgagccactt 60
modified atgtcacattctaccttagtccccatggtccaaggctactgagaccatgagccactt 60
*****
origina gtaacaccaacctogaacaagattaacggtgaatattgtacctggctcaagagctat 120
modified gtaacaccaacctogaacaagattaacggtgagattctgctggctcaagagctat 120
** *
origina accaatcgagctctaactcattgctgcttttagcagagggactctacacttaaggaata 180
modified accaacgcgctctcatctattgctgctttggccaggggactctacaccttaaggaatc 180
*****
origina ttaagagtgatgatctactggtgattgatgccttaaggagcttggcattaaagatc 240
modified tgaagagtgatgatctactggtgattgatgccttagagagcttggcattaaagatc 240
** *
origina gaggtgcccgaagagcgtcaccattcatggctgtggaggaaatggcagttcaatct 300
modified gaggtgcccgaagagaccgtgaccattcatggctgcccggaggaaatggcagttcaatct 300
*****
origina gcagagcttttattgggctgcaggtaccattgcccttccctccaggagccttagct 360
modified gccagagcttttattgggctgcaggtaccattgcccttccctccaggagccttagct 360
** *
origina gttgccagcaagggagtgatgtagatggggtccacaactgcgagaagaccatta 420
modified gttgccagcaagggagtgatgtgagatggggtccacaactccgcgagagaccattg 420
*****
origina aaacctttagtgatgcttaactacgcttgggtagaatagagatctgactgagcat 480
modified aaacctttagtgatgcttaactacgcttgggtagaatagagatctgactgagcat 480
** *
origina ccgggtctgctttacgagtaaaaggggcaaggtctaaagtgacacatgtaaggggccca 540
modified ccgggtctccctttgcgcgtgaaggggagctggctcactggagacacatgtaaggggccca 540
*****
origina ggaatgtctctagccaattttaagtgtttattaatgccagctctatgctcagaa 600
modified ggaacgtgtctagccaatcttgatgtttgttgatgccagctctatgctcagaa 600
*****

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origina      gctgtcagcattgaggtaatcaatggactcgttcaaccgcttaccattgccattacgatt      660
modified    gctgtgagcattgaggtgatcaacggactcgttcaaccgcttaccattgccattaccatt      660
*****

origina      cagttaatgagagaattgggccaaagtgagcataatgaggttacagctctcttaag      720
modified    cagttgatgagaggttcgggtccaagtgagcataaacgaggttacagctctctcaag      720
*****

origina      gttaccctactggataccaaggtcgtgataccatctgaggcagatgctcaacagcc      780
modified    gttaccctactggataccaaggtcgtgataccatctgaggcagatgctcaacagcc      780
*****

origina      tgcattttctatccttagcgcgttaactggaggtaccatccaggtgaagaatgtggc      840
modified    tgcattctctcctcctggccggttgactggaggtaccatccaggtgaagaatgtggc      840
*****

origina      taccattcgtaccagcagatgctcgtttcattgatgtgttagagaaatggcgtgaa      900
modified    taccattcgtaccagcagatgctcgtttcattgatgtgttagagaaatggcgtgag      900
*****

origina      gtgattaagaatgagtcattcctagaggttacagcccaaccgattaaaggtggcttc      960
modified    gtgattaagaatgagtcattcctagaggttacagcccaaccgattaaaggtggcttc      960
*****

origina      gaggtggatagaagcctatgctgccaagcgttgaccataggcattagctcctttt      1020
modified    gaggtggatagaagcctatgctgccaagcgttgaccataggcattagctcctttt      1020
*****

origina      gcagatgcaccgattcgggtaaccaatgctcgtcacattaggctcatgagtcagaccg      1080
modified    gccgatgcccgattcgggtgaccaatgctcgtcacattaggctcatgagtcagaccg      1080
*****

origina      atagctgtattgttctcctgttacagcagatgggagttcaggtagagagagagagat      1140
modified    atcgcgttattgtcctcctgttacagcagatgggagttcaggtagagagagagagat      1140
*****

origina      ggcttactatctacaggtcagcagtggtacaacgcttaactcctcatgatgatcat      1200
modified    ggcttactatctacaggtcagcagtggtacaacgcttaactcctcatgatgatcat      1200
*****

origina      cgtaatgcaatggtattcgttacttggagtaaaagtaccacatattagaatgctgac      1260
modified    cgtaaogccatggttcggttcttggagtaaaagtaccacatattagaatgctgac      1260
*****

origina      ccgggtgtgtatctaagacctgccagcctatttgaagagctgcagaagttggaata      1320
modified    ccgggtgcgtgtctaagacctgccagcctatttgaagagctgcagaagttggaata      1320
*****

origina      catgtggagtataat      1335
modified    catgtggagtaaac      1335
*****

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- Way of transgenicity: Agrobacterium-mediated transformation:

I broke that into two phases.

- A- Plasmid construction (insert wanted sequence -n=1335- into the T-DNA assigned sequences). the plasmid has many segments that are called VIR + a variable letter w/w/o number. Each VIR products facilitate the T-DNA sequence into the plant cell, maize in my case. In addition to VIRs, there are different segments (for example: traR) that I haven't look into what they do due time restrains-sorry-.
- B- T-DNA delivery to maize genome.

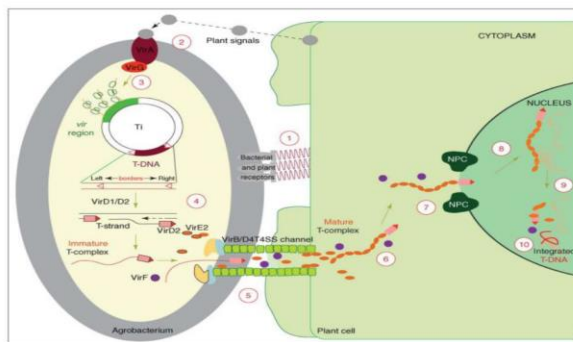


Figure 2.5: Process of *Agrobacterium* mediated transformation in a plant cell. (1) Recognition and attachment of the *Agrobacterium* to the host cell; (2) The sensing of specific plant signals by the *Agrobacterium* VirA/VirG; (3) The *vir* gene region is activated; (4) A mobile copy of the T-DNA is generated by the VirD1/D2 protein complex, and delivered as a VirD1-DNA complex (immature T-complex); (5) The immature T-complex and several other Vir proteins are delivered into the host cell's cytoplasm; (6) The mature T-complex formed when VirE2 is associated with the T-strand, then travels through the host-cell cytoplasm; (7) T-complex is actively imported into the host-cell nucleus; (8) inside the nucleus, the T-DNA is recruited to the point of integration; (9) T-DNA, stripped of its escorting proteins; (10) Finally, the DNA was integrated into the host genome. (Adapted from Tzifira *et al.*, 2004)

- Mechanism of action: glyphosate INHIBITS EPSPS that mediate PEP → EPSP and S3P → EPSP. i.e. it kills the plant by blocking their metabolite activity. (acts on Shikimate Pathways).

III. Regulatory issues

Glyphosate resistance maize mineral content? No effect on mineral content (Reddy, 2018).

V. REFERENCES (there are more articles in here because my outline contains the sum of knowledge I got from -mostly- the following articles)

- Chikoye, Schulz, & Ekeleme. (2004). Evaluation of integrated weed management practices for maize in the northern Guinea savanna of Nigeria. *Crop Protection*, 23(10), 895-900.
- Ren, Zhen-Jing, Cao, Gao-Yi, Zhang, Yu-Wen, Liu, Yan, & Liu, Yun-Jun. (2015). Overexpression of a modified AM79 aroA gene in transgenic maize confers high tolerance to glyphosate. *Journal of Integrative Agriculture*, 14(3), 414-422.
- A. Razak, Roslinda. (2018). Agrobacterium-mediated transformation of *Carica papaya* L. var Eksotika with improved resistance to dieback disease. 10.13140/RG.2.2.24985.11367.
- Hetherington, P., Reynolds, T., Marshall, G., & Kirkwood, R. (1999). The absorption, translocation and distribution of the herbicide glyphosate in maize expressing the CP-4 transgene. *Journal of Experimental Botany*, 50(339), 1567-1576.
- Dun, Baoqing, Wang, Xujing, Lu, Wei, Chen, Ming, Zhang, Wei, Ping, Shuzhen, . . . Lin, Min. (2014). Development of highly glyphosate-tolerant tobacco by coexpression of glyphosate acetyltransferase gat and EPSPS G2-aroA genes. *The Crop Journal*, 2(2-3), 164-169.
- Reddy, K., Cizdziel, J., Williams, M., Maul, J., Rimando, A., & Duke, S. (2018). Glyphosate Resistance Technology Has Minimal or No Effect on Maize Mineral Content and Yield. *Journal of Agricultural and Food Chemistry*, 66(39), 10139-10146.