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VCE Biology ¾

AOS 1 Revision [1.0]

SAC 9 Solutions

40 Marks. Y Minutes Reading. Z Minutes Writing.

Section A: SAC Questions (40 Marks)

Question 1 (40 marks)

Read the article and answer all questions in the spaces provided.

Control of Bacterial Disease in Banana using CRISPR-Cas9

Adapted from an article by Leena Tripathi, Valentine Ntui and Jaindra Tripathi, sourced from The National Library of Medicine 8/12/22 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8998688/

Banana is an important staple food crop and source of income for farmers in 136 countries around the world. Annual banana production in 2021 was over 163 million tons, one third produced in Africa, where it provides 30-60% of the daily per capita calorie intake.

Banana Xanthomonas Wilt (BXW) is an easily spread **bacterial disease** that can affect all species of banana cultivated by humans. Its effects are severe and swift, **wiping out whole plantations**, with economic losses over the last decade estimated up to US $8 billion.

Developing disease-resistant varieties of bananas using traditional breeding methods is challenging for many reasons, including the low genetic variability in banana species. Other measures to prevent the transmission of the disease are very labour intensive, such as using sterile gardening tools and burying infected plants. Consequently, significant efforts have been made to produce bananas with resistance to BXW through genetic manipulation.

When a banana plant is **infected with BXW**, expression of a gene called Downy Mildew Resistance 6 (DMR6) increases, leading to the **suppression of the plant's immune function** (its ability to fight disease). Scientists in Kenya have recently used CRISPR-Cas9 technology to decrease the expression of DMR6 in greenhouse trials, creating bananas resistant to BXW. However, the commercialisation of genetically modified crops is hampered by lengthy regulatory procedures.

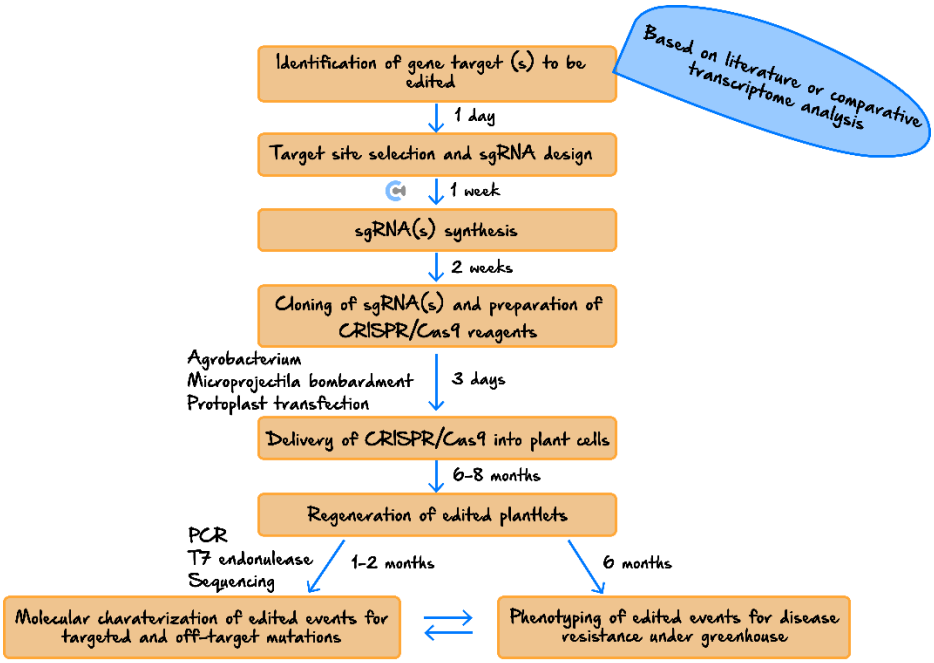


Figure 1: Flowchart illustrating the steps and approximate time needed to develop gene-edited banana

a. Explain how genetic editing of the DMR6 gene using CRISPR-Cas9 can increase the yield of banana plants. (2 marks)

Decreased expression of DMR6 gene.
Immune function not suppressed when infected by BXW.

b. Other than increasing food supply, suggest another benefit to humans of editing the DMR6 gene in bananas. (1 mark)

Increased income for farmers or other suitable response.

c. The article states that "commercialisation of genetically modified crops is hampered by lengthy regulatory procedures." Editing of plant genomes raises a range of ethical issues.

i. State the cause and effect of an ethical concern related to the editing of plant genomes. (2 marks)

Sample response only: ***SEE BELOW TABLE FOR MORE EGS.***
Cause: Off-target mutations can occur.
Effect: Affecting the function of required genes causing unintended consequences.

ii. Discuss how a **consequences-based approach** could be applied in the evaluation of the use of CRISPR-Cas9 technology to edit the genomes of banana plants from the perspective of a research scientist. **Include your decision** as to whether the technology should be used on bananas. (3 marks)

Sample response only:
Consequences-based:

- Positive: Increased yield and income resulting from a scientist's work are the aims of his research, ensuring professional success/continued employment.
- Negative: Off-target mutations and adverse immune responses would lead to less public trust in the products of gene editing, less investment in research and fewer employment opportunities for the scientist.
- Positive consequences outweigh the negative, as more people stand to benefit from the technology than would be potentially harmed. Therefore, the use of CRISPR on bananas is ethically acceptable.

1m for potential positive consequences relating to a research scientist.
1m for potential negative consequences relating to a research scientist.
1m for decision based on a comparison of consequences.

d. Outline how any one ethical concept should be taken into account by scientists when using CRISPR-Cas9 technology to modify the genome of food crops. (1 mark)

Sample response only: (Must include the name of the concept.)
Integrity: Honest reporting of results of all trials, both positive and negative.

The expression of the DMR6 gene eventually results in the production of a protein called 2-oxoglutarate Fe[II] dependent oxygenase - or 2OGO. The 2OGO protein is made of one polypeptide, 341 amino acids long; its 3D structure is shown below.

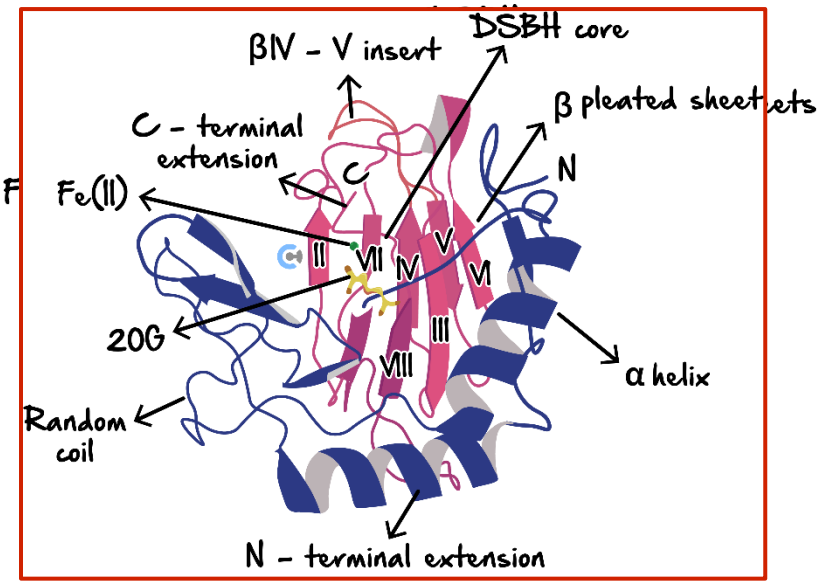


Figure 2: 3-dimensional structure of 2-oxoglutarate Fe[II] *dependent oxygenase* (2OGO)

e. There are elements of the secondary structure of the 2OGO protein evident in the diagram above. Clearly label the diagram with the names of any two of these elements. (2 marks)

f. The end product of gene expression is a polypeptide. Describe how the polypeptide resulting from the expression of the DMR6 gene differs from the final 2OGO protein. (3 marks)

- The polypeptide is just a chain of amino acids.
- 2OGO has secondary structure, as the polypeptide folds locally into a helices and β pleated sheets.
- 2OGO protein has tertiary structure, as the polypeptide folds on itself into a 3D shape.

g. Is 2OGO part of the banana plant's genome or proteome? Explain. (1 mark)

2OGO is part of the proteome as it is one of the sets of all proteins made by the banana's cells.

h. Gene expression in banana cells is essentially a two-stage process.

i. The first stage is transcription in the nucleus, which produces a primary mRNA transcript. This molecule is then modified to produce mature mRNA, which leaves the nucleus. Briefly describe the three main ways that the mRNA transcript is modified in banana cells. (3 marks)

Introns removed.
Methyl cap added to 5′ end.
Poly *A* tail added to 3′ end.

ii. The second stage of gene expression occurs in the cytoplasm of banana cells. Briefly describe the involvement of any nucleic acids in the production of the DMR6 polypeptide during this stage. (3 marks)

Translation involves the following nucleic acids:

- Ribosomal RNA makes up ribosomes, the sites of translation.
- Messenger RNA carries a copy of the DNA code to the ribosomes.
- Transfer RNA carries specific amino acids to the ribosomes to make the polypeptide.

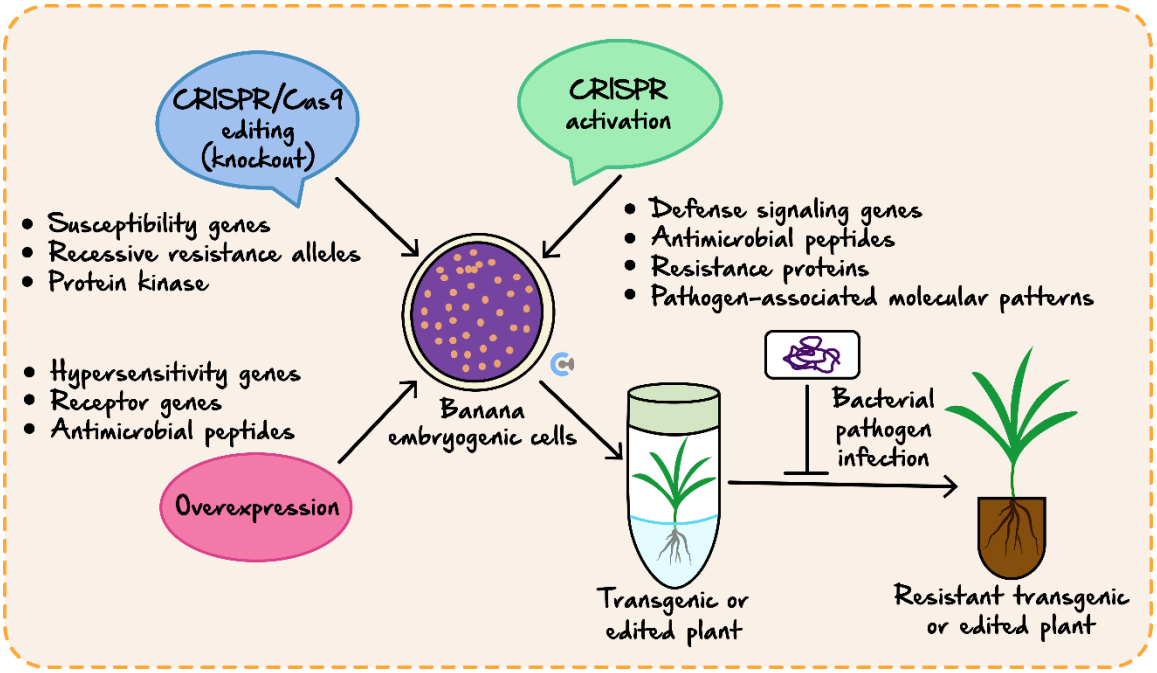


Figure 3: Schematic diagram illustrating strategies for developing bacterial disease resistant banana varieties.

The scientists used CRISPR-Cas9 technology to edit the DMR6 gene in embryogenic banana cells (the first cells or embryo that will develop into a banana plant) with a targeted mutation, causing a gene "knockout". They aimed to delete a section of the gene 114 base pairs (bp) long from a region in exon 4. To do this, they needed to create two separate sgRNA molecules, one for either side of the segment to be deleted, leading to two simultaneous cuts, 114bp apart. These sgRNA molecules were then delivered to embryogenic banana cells.

i. Why did scientists target an exon rather than an intron? (2 marks)

Introns are non-coding segments of DNA deleting an intron may not silence the gene.

j. Briefly describe how scientists would have decided on the sequence of the sgRNA molecule. (1 mark)

Each would be synthesised complementary to a sequence either side of the 114bp target.

k. Deletion of a region in exon 4 would cause a truncated (shortened) polypeptide to be produced. Suggest a way that scientists could induce a different deletion to cause a total lack of DMR6 expression. (2 marks)

Deletion of a region upstream of the gene to remove the promoter region prevents RNA polymerase from binding so transcription doesn't begin.
Or other suitable response that would lead to no transcription.

Scientists also needed to insert the Cas9 gene into the embryogenic banana cells. A plasmid was used as a vector. Once in the banana cells, the Cas9 gene is integrated into the banana genome.

l. Describe how a restriction endonuclease and DNA ligase would have been used to insert the Cas9 gene into the plasmid. (3 marks)

Restriction endonuclease cuts at specific sequences either side of the Cas9 gene.
Same restriction endonuclease cuts same sequence in plasmid to create **complementary sticky ends**.
DNA ligase joins the **sugar-phosphate backbones** (or creates phosphodiester bonds) of the plasmid and gene.

m. What was the purpose of introducing the Cas9 gene to the banana cells? (2 marks)

Cas9 gene produces Cas9 endonuclease.
Cas9 is needed to cut the DMR6 gene to achieve the gene knockout.

n. Outline the function of sgRNA in the editing of the gene. (1 mark)

Guides Cas9 to the target sequence where it will cleave DNA.

o. Are the banana plants produced by gene editing transgenic organisms? Explain. (1 mark)

Yes, as their genome contains the Cas9 gene from bacteria.

After CRISPR-Cas9 treatment, the embryogenic banana cells were allowed to develop into plantlets (small plants). Scientists discovered a 100% mutation rate in the 5 resulting plantlets. They confirmed the targeted mutations by amplifying the target region in a thermocycler and then sequencing it. The plantlets were then potted and allowed to grow for 90 days; there were no differences detected in plant height or leaf area in the 90-day-old mutant plants compared to the unedited plants.

p.

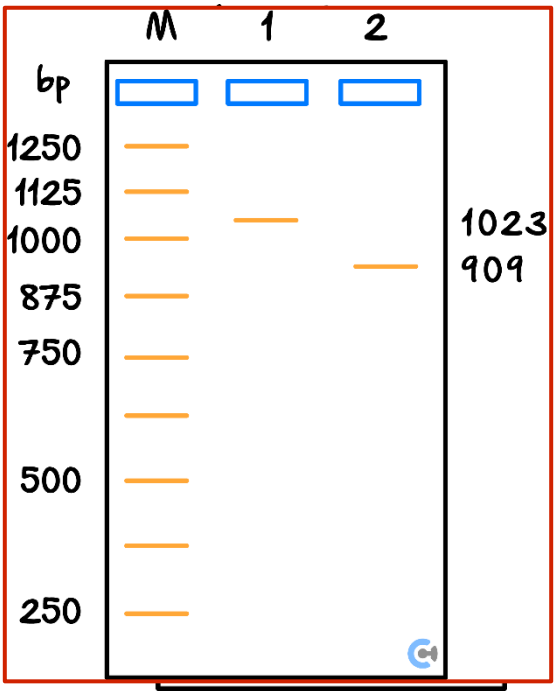
i. Name the process that enabled the scientists to amplify DNA. (1 mark)

Polymerase Chain Reaction

ii. Instead of DNA sequencing, gel electrophoresis is another technique that can be used to detect a deletion in a gene. Explain how the process of gel electrophoresis works to enable the detection of a 114 base pair deletion. (4 marks)

DNA containing the gene is inserted into wells of the gel
Electric current is applied.
Negatively charged DNA migrates to the positive electrode.
The smaller fragments of DNA with the deletion move more easily through the gel than larger fragments without the deletion.
Or A DNA ladder is used to check for the fragment that is 114bp shorter.
Must refer to how the deletion is detected.

iii. If mature mRNA molecules from the expression of the DMR6 gene of both unedited and edited plants were run on a gel, draw on the diagram below where the resulting bands would end up. (Use Lane 1 for unedited and Lane 2 for edited RNA.) (1 mark)



q. Scientists collected evidence from both edited and unedited banana plants to show that growth of the plants was not impacted by the editing process. From the information on the top of the previous page (pg 9), choose a limitation of one element of the evidence and briefly explain how it could be improved. (1 mark)

One of:

- Examine more than 5 plantlets to increase sample size/reliability.
- Continue to monitor the plant growth for more than 90 days, as problems may emerge later.
- Examine other health markers in addition to plant height and leaf area to ensure other aspects of growth are not negatively impacted.

Or other suitable response.

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