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Synthetic Spider Silk Protein in Alfalfa

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Spider Silk

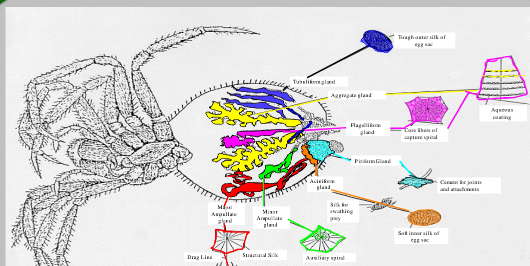

Figure 1: Diagram of spider silk glands.

Over the past 400 million years, orb-weaving spiders have paved a genetic pathway to create a biomaterial with properties that surpass those of most man-made materials: spider silk (Figure 1). Spider dragline silk has high tensile strength and elastic properties making it of interest for parachute chords, tendon and ligament replacements, sutures, and body armor. Synthetic spider silk production has been successful in E. coli, yeast, silkworms, and goats, but yields remain too low for spider silk to be commercially viable. Thus, there is a need for a large-scale production method like plants.

Why Alfalfa?

Alfalfa is a perennial crop used in agriculture as forage for livestock because of its high yielding nutritional value and protein levels that reach up to 24% per dry weight. Alfalfa transformation with *Agrobacterium tumefacians* has been achieved, creating transgenic alfalfa with silk protein. By inserting the gene construct, major ampullate silk protein 2 (MaSp 2), for spider silk into alfalfa a silk protein yield of up to 2% could be achieved, making spider silk production commercially viable. After the spider silk protein is purified from the alfalfa leaves, the remaining plant tissue is waste. The waste from the spider silk purification process can be used as livestock feed or for producing ethanol, removing the cost of waste disposal and increasing the appeal for using alfalfa.

Transgenic Alfalfa

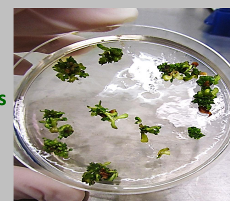

Figure 2: Callus tissue transformed by *Agrobacterium tumefaciens* with the spider silk protein gene. As callus develops on nutrient-rich agar, leaflets and small roots will begin to form.

Figure 3: Transgenic alfalfa with a developed root system. Once plant size increases the plant will be transplanted into soil. The leaves are harvested and the MaSp2 protein extracted.

In order to extract and purify the synthetic spider protein in the transgenic alfalfa the following steps must be taken:

- The leaves must be harvested and homogenized in 8 M Urea.
- After centrifugation the decanted solvent is processed using a nickel chromatography column on the AKTA.
- Elutants with the spider silk protein are dialyzed and lyophilized creating a protein powder.

Results

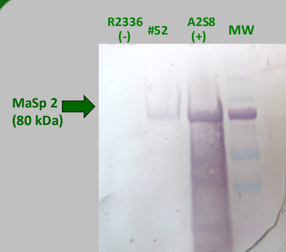

Figure 4: Western blot showing a purification of the native R2336 alfalfa (-), a 10g purification from Plant #52, the synthetic MaSp2, A2S8 (+), and the molecular weight (MW) marker

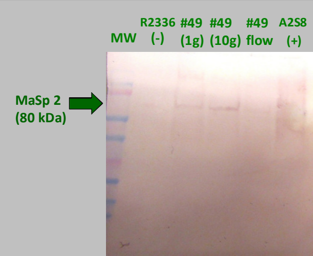

Figure 5: Western blot showing the MW, R2336 (-), a 1 g purification from Plant #49, a 10g purification from Plant #49, Plant #49's flow through, and A2S8 (+).

Discussion

We have successfully agro-transformed the 2 X MaSp2 construct into alfalfa and can extract the protein(Figures 4 and 5). The magnitude of spider silk expression is high enough that the protein can be detected on a 1 g scale (Figure 5). This is important because it allows earlier detection of successful transformation, and also implies a high protein yield.

Table 1: The spider silk protein yield produced by each synthetic process.

System	Protein Yield
Bacteria	3 kg per 30,000 L
Goats	18 kg per goat per year
Alfalfa	218 kg per acre
Silkworm	unlimited

A 1% spider silk protein yield in alfalfa creates a commercially viable process, with a much higher yield than any other system (Table 1). Further work is needed to determine the spider silk protein yield in alfalfa.

Summary

Currently we have agro-transformed alfalfa to produce and express spider silk protein. Further research will determine if the silk protein expression is high enough for commercialization. Future work will concentrate on scaling up production and modifying our constructs to express larger spider silk proteins.

Contributors

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