Progress towards transformation of fibre hemp

L. MacKinnon, G. McDougall, N. Aziz & S. Millam

Introduction The last decade has seen the reintroduction to the UK of a crop with a history of triumphs and tragedies; today its very name provokes visions of a drug culture, fuelled by its prohibition in Britain for over 70 years. Historically, cultivation of *Cannabis sativa* L. (hemp) originated in China around 2700 BC, where its properties as a medicinal plant were first discovered. Cultivation then spread across Asia and through Europe, arriving in the UK some 2000-2200 years ago, by which time it had become widely cultivated as it had so many uses. Hemp fibres were found to be durable and were used in clothing, sailmaking and papermaking. Notably, the first copies of the Bible were composed of hemp paper. Oils from hemp seeds were used for a wide range of purposes, from cooking to cosmetics, and extracts of hemp were used to treat a wide range of ailments. Queen Victoria is rumoured to have used hemp on a regular basis. This widespread industrial use of hemp continued to the early 20th century, until cheap and plentiful imported jute and cotton made hemp uncompetitive. Around this time, as the legitimate uses of hemp declined, the misuse of hemp as a hallucinogenic agent became more apparent and many countries began to outlaw the cultivation of cannabis. In 1928, an act was passed that finally prohibited hemp cultivation in the UK.

Hemp cultivation continued in other European countries, such as The Netherlands and France, and in many Russian states. European hemp breeding programmes produced hemp cultivars for fibre production that were low in the psycho-active constituents, cannabinoids. Eventually, cultivars with ultra-low (<1% wild type) cannabinoid levels were available, which reawakened UK interest in hemp. In 1994, a new hemp-licensing scheme allowed the crop to be cultivated again in the UK and its reintroduction began. Over recent years, hemp cultivation has been increasing steadily (Fig. 1). This renaissance is also attributable to the need for renewable non-food crops in the UK, as outlined by the UK Biodiversity action plan.

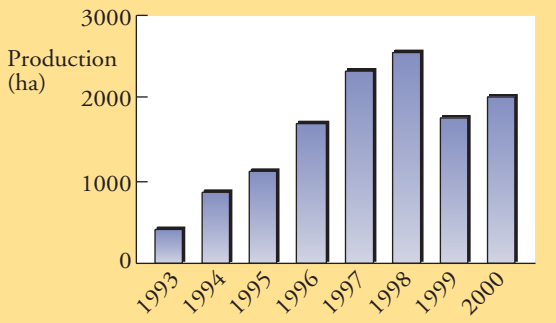

Figure 1 The UK production of hemp over recent years.

Why grow hemp? Hemp is a particularly environmentally friendly crop and can be grown on soils poor in nutrients, with no additions of fertilisers. Routinely, it needs no herbicide or pesticide inputs and can be grown on set-aside land. It has been found to be useful in crop rotations as it suppresses three major soil pathogens, reducing the need for pesticide use in subsequent crops.

Hemp has a short growing season, reaching maturity in only 100 days, which makes it a useful alternative annual crop, and there is a current EU subsidy of 100 euros per tonne of hemp produced in Europe.

In addition to the environmental aspects, hemp is a multi-use crop and can provide fibres for paper, board and textiles; oil from the seeds; and provide stem residues (straw), which are valued as non-edible bedding for animals.

One of the main applications of hemp is for fibres for the papermaking industry, and there has been a great deal of research in this area. Unlike other fibres such as cotton, which require considerable processing and strong chemical treatments prior to use, hemp fibres are obtained by retting in water and can be bleached prior to use with hydrogen peroxide, which produces only water as a by-product. In addition, there is scope for hemp fibres to be used in the high quality textile industry.

Why transform hemp? Despite its many natural advantages, hemp could be improved further as a valuable multi-use crop. There is a need for new hemp products so that the crop can achieve long-term competitiveness, even as an alternative or rotational crop. The use of genetic transformation to produce novel products is a possible route. Additionally, although hemp suppresses certain soil pathogens, as discussed above, it is susceptible to infestation by *Botrytis cinerea* and significant losses, which are not prevented by pesticide use, can result. Transformation of hemp with existing genes that increase plant resistance to fungal infection may be an answer to this problem.

The properties of the fibre could be improved to match consumer requirements. For example, transformation of hemp with genes involved in lignin biosynthesis, could be used to upgrade fibre quality and widen potential end-uses in the paper and textile industries. Genetic modification of the oil profile of hemp seed, as achieved for oilseed rape, could find high-value niche markets.

Such improvements in hemp quality could be achieved by conventional breeding techniques. However, conventional breeding is a very slow process, which could take decades, especially for *Cannabis* which has not been intensively bred. An additional problem is that the existing, legally acceptable fibre hemp cultivars, have been bred to be low in cannibanoids and breeders must ensure that this trait is not bred out.

Towards the transformation of hemp There has been very little work on the biotechnology of hemp[1] and therefore basic research was required to establish tissue culture and regeneration systems suitable for the crop. Two French hemp fibre cultivars (Fedora 19 and Felina 34) were selected for use in this project. Both have low cannabinoid contents and are genetically similar, but Felina is an early maturing genotype. These cultivars were obtained from an accredited source (Hemcore) and are grown under Home Office licence.

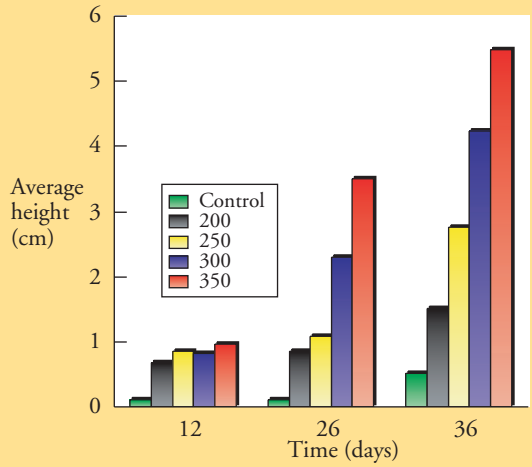

Figure 2 The average height of Felina shoot tips grown under varied concentrations of cefotaxime.

Establishment of tissue culture and regeneration systems The establishment of effective tissue culture and regeneration systems is an essential prerequisite of genetic transformation for any plant species. A large series of experiments was undertaken to establish sterile plants in tissue culture, regeneration of explants, and shoot tip regeneration. Effective protocols have been established for growing hemp seedlings *in vitro*, with the different varieties having different media preferences. Callus and roots were easily obtained from hemp explants but shoots were not readily produced. Thus, alternative transformation strategies were devised and, as a result, a method of transformation involving shoot tips was developed from methods previously reported in cotton[2] and petunia[3].

It was found that shoot tip regeneration was markedly improved by the addition of antibiotics, specifically cefotaxime (Fig. 2), and subsequent shoot tip growth was aided by reduced light treatment.

Susceptibility of hemp to infection by the genetic transformation agent *Agrobacterium tumefaciens* The most common and convenient means of introducing genes into plants is to use the bacterium *Agrobacterium tumefaciens* as the vector. However,

Figure 3 Stem section of *C. sativa* infected by wild-type *Agrobacterium.*

since the ability of this bacterium to infect hemp had never been reported, experiments were undertaken to confirm its potential as a transformation agent. Over 50% of hemp plants exposed to this bacterium exhibited the classic Crown gall (Fig. 3), which confirmed that genetic transformation of hemp using this vector should be possible. An efficient transformation system for hemp has now been developed.

Botrytis-resistant hemp As mentioned previously, hemp is susceptible to infection by the fungus, *Botrytis cinerea*. Previous work at SCRI has shown that polygalacturase inhibitory proteins (PGIPs) can convey resistance to this disease[4]. PGIP genes were introduced into hemp, along with herbicide resistance as a selective marker of transformation. The efficiency of our transformation method was comparable to other crop efficiencies. Initial molecular analysis has confirmed that the transformed herbicide-resistant plants also contain the PGIP gene. The PGIP positive transformed plants have been tested for resistance to *Botrytis* and preliminary results (see figure 4) are encouraging.

Figure 4 Plants 1-4 are transformed with an empty vector control plasmid, plant 5 transformed with PGIP genes. Photo taken 3 days post-inoculation with *Botrytis cinerea.*

Conclusions A reliable and effective transformation system has been developed. This is the first report of genetic transformation in hemp. This preliminary work will enable more targeted approaches to gene transfer in this valuable crop species in the future and enable the range of uses of this versatile and historically significant plant to be enhanced.

References

[1] Ranalli, P., DiCandilo, M., Mandolino, G., Grassi, G. & Carboni, A. (1999). *Agro-Food-Industry-Hi Tech* 2, 33-38.

[2] Zapata, C., Park, S.H., El-Zik, K.M. & Smith, R.H. (1999). *Theoretical and Applied Genetics* 98, 252-256.

[3] Ullian, E.C., Smith, H., Gould, J.H. & McKnight, T.D. (1988). *In Vitro Cellular and Developmental Biology* 24, 951-954.

[4] Johnston, D.J. & Williamson, B. (1994). *Journal of Experimental Botany* 45, 1837-1843.