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RESEARCH HIGHLIGHT

Need for speed: a breakthrough speed breeding protocol for hemp

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Archeological evidence suggests that the cultivation and use of cannabis (*Cannabis sativa*) can be traced back almost 12 000 years (Crocq, 2020). Chinese legends state that the emperor Shén Nóng discovered the medicinal properties of cannabis as far back as 2000 BCE. The Ebers papyrus, written in Egypt around 1500 BCE, describes the anti-inflammatory properties of cannabis, while the Roman historian Pliny the Elder wrote about cannabis in the first century CE, mentioning its medicinal effects as well as its importance in providing fiber for rope making.

Today, most of the discussion around cannabis revolves around its psychotropic and potentially medicinal properties, but it is important to note that cannabis has a myriad of other beneficial uses. As noted by Pliny, fibers derived from the cannabis plant have been used for millennia, so much so that the English word 'canvas' is derived from 'cannabis'. Cannabis can also be grown for food, as its seeds are high in protein, fatty acids, vitamins, minerals, and fiber (Cerino et al., 2021). Modern technology has unlocked even more uses for this multifaceted crop, since cannabis-derived plastics and even concrete have been developed (Johnson, 2018).

However, due to the intoxicating effects of cannabis, it was criminalized in the 20th century. The legal history surrounding the prohibition of cannabis is complicated, but the United States was the first country where its growth and possession were outlawed, in 1937 (Collins, 2020). In 1961, the UN Single Convention effectively banned psychotropic cannabis across the globe. Growing cannabis as an intoxicant remains illegal in most countries, but in recent decades growing non-psychotropic cultivars (referred to as 'hemp') for use as a food and fiber crop has gained renewed attention. Nevertheless, due to this almost universal ban, there is a lack of fundamental research into this controversial plant.

Although this significant dearth of knowledge might be seen as an impediment to research, Susanne Schilling saw it as an opportunity. After completing a post-doc investigating molecular evolution of the MADS-domain protein family in wheat (*Triticum aestivum*), Schilling was offered a position working with Rainer Melzer and Paul McCabe at University College Dublin in Ireland. The main focus of their project was to modify a set of speed breeding methods developed in wheat and other long-day crops (Watson et al., 2018) to facilitate and accelerate hemp breeding.

One of the few aspects of cannabis biology that was well understood is that it is a short-day plant. In fact, the concept of photoperiodism was first defined in 1912 thanks to work in cannabis (Tournois, 1912, as cited in Kobayashi & Weigel, 2007). Because of this, Schilling needed to figure out a way to modify the speed breeding protocol that had been developed for long-day plants. She started by growing two photoperiod-sensitive hemp varieties along with a photoperiod-insensitive variety under artificial light in a temperature-controlled environment, through a complete life cycle (Schilling et al., 2023).

All of the plants were grown for 2 weeks under continuous light in order to promote maximum biomass accumulation; then, subsets were grown under four different light regimes for 60 days: ultra-short days (8:16), short days (12:12), long days (16:8), and continuous light (24:0). Unsurprisingly, the photoperiod-insensitive cultivar did not display any difference in flowering time under different light regimes. The photoperiod-sensitive plants flowered much sooner during short days, although there was little difference in flowering time between those grown in short or ultra-short days. Most of the photoperiod-sensitive plants grown in continuous light did not flower during the 60 days, and some of the plants grown in long days also did not flower. The authors note that ultra-short days did impact the plants physiologically, as these plants were shorter and feebler than those grown under the other regimes.

Schilling next decided to modify the initial growth period under continuous light. Shortening this period from 14 to 7 days did not shorten flowering time, while eliminating it increased flowering time. She also tried growing the plants in smaller pots under the assumption that the stress of being pot-bound would accelerate flowering, but, contrary to her expectations, this increased flowering time.

In order to assess if these initial findings would be broadly applicable, Schilling grew seven additional hemp cultivars under the same conditions, i.e. 2 weeks of continuous light followed by short days. Even though these seven genotypes included landraces as well as industrial cultivars, the flowering times of all seven were synchronized, ranging from 29.2 to 33.3 days. This is especially remarkable considering

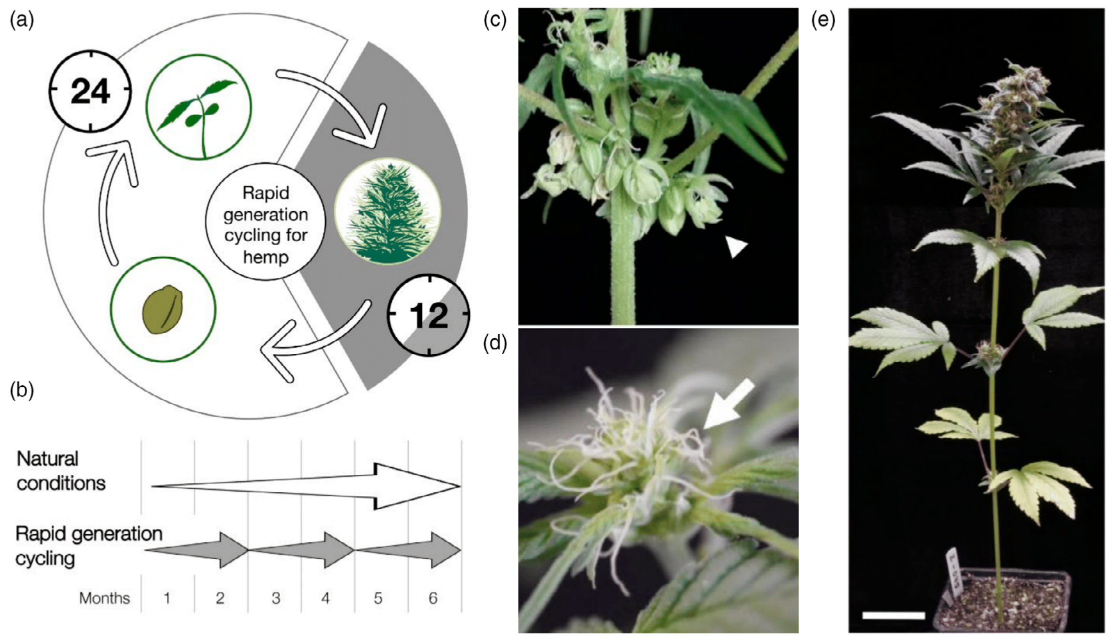

Figure 1. Overview of rapid generation cycling (speed breeding) in hemp.
(a) Seeds are incubated for 2 days in darkness. Seedlings are then grown for 2 weeks under continuous light (24). Plants are grown under short-day conditions (12) for 4 weeks to induce flowering, pollination, and seed setting. Plants are then grown under continuous light to limit further flowering and accelerate seed ripening. Water stress is introduced in week 8 to accelerate seed ripening.
(b) By using this protocol, three generations of hemp can be grown in the time it would normally take a single generation.
Male [(c), arrowhead] and female [(d), arrow] hemp flowers.
(e) A hemp plant grown under speed breeding conditions has ripening seeds after approximately 60 days. Modified from Schilling et al. (2023).

that flowering times among these genotypes under natural light conditions range from 50 to 130 days. These results suggest that these conditions to shorten the life cycle ought to be a viable option for hemp breeders, regardless of genotype.

However, accelerated flowering time alone does not help breeders if the resulting seeds are not viable. Luckily, Schilling observed germination rates of 55–86%. She noted that seed set can also be accelerated by subjecting the plants to continuous light and withdrawing water after they are pollinated. Using this method (Figure 1a), Schilling achieved a seed-to-seed generation time of 61 days, which is a third of the time needed for plants grown in the field (Figure 1b). Although she admits that there may be ways to further optimize this protocol, for example by using hormones to increase flower and seed density, Schilling thinks that 61 days is probably close to the minimum generation time in hemp, since the plants require about 2 weeks of vegetative growth before flowering. She hopes this protocol will radically accelerate genetic research on hemp, for example for developing mapping populations. This future genetic research could focus on maximizing biomass accumulation for biofuel production, modifying fiber quality or other characteristics relevant to industry, or increasing the quantities of non-psychotropic but potentially medicinal secondary metabolites.

Schilling and her co-authors Melzer and McCabe are continuing to research hemp genetics. Specifically, they are working on flowering time control, flower development, sex determination, and a genome-wide analysis of transcription factors. As for the current lack of experimental knowledge about hemp, Schilling is optimistic about filling in those gaps. She has a wealth of previous plant biology research in other species to build on, and she is confident that insights gained from other plants can be applied to hemp research.

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