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Conceptual Paper

Merging Biotechnology with Non-Food Plants for Medicinal Purposes

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Agriculture is approximately a 10,000 years ago human practice and is estimated that more than 7,000 species are to satisfy basic human needs [1]. The primitive crop cultivars were adapted to local growing conditions and practices, and therefore remained genetically diverse for traits such as product qualities, stress tolerance, disease resistance, and yield stability. By contrast, modern crop cultivars are more genetically uniform than their wild relatives [2]. Nowadays, biodiversity is increasingly being recognized as a vital resource for economic, social and environmental development. Plant germplasm provides the raw materials we rely upon for food, fiber, energy, medicinal and industrial products. Plant genetic diversity increases options and may provide innovative, plant-based solutions to the major environmental challenges that we all face - food security, water scarcity, deforestation, energy and climate change.

The industry is expected to supply high-quality products while remaining sustainable and cost-efficient. The nowadays affordable high throughput DNA sequencing, coupled with improved bioinformatics and statistical analyses, is bringing major advances in the field of molecular plant breeding. Multidisciplinary breeding programs are able to investigate genome-wide variations in DNA sequences and link them to inherited highly complex traits which are controlled by many genes, such as hybrid vigor and flowering. Among other demands, agriculture is trying to sustainably produce high quality crops for medicinal purposes. The progress in molecular plant breeding can help meet these demands by shortening new crop domestication time, tailoring existing crops to meet new requirements, such as nutritional enhancement or climate change, and rapidly incorporate valuable traits from wild relatives into established crops.

Genomic resources

Coneflower (Echinacea angustifolia DC)

Echinacea species are members of the Asteraceae family and include *E. angustifolia, E. pallida, E. simulata, E. paradoxa, E. tennesseensis, E. laevigata, E. sanguinea, E. atrorubens, E. gloriosa,* along with *E. purpurea*. However, only three species of *Echinacea* are generally used medicinally: *E. purpurea Moench* (roots and tops), E. angustifolia DC (roots) and E. pallida Nutt (roots). Different types of DNA-based markers viz., RAPD, RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat) etc. are employed for plant species discrimination coupled with methods of plant identification involving taxonomy, physiology and embryology. AFLP molecular markers have been developed [3] and retrotransposon sequences [4] have been used to fingerprint and study the genetic diversity among *Echinacea* taxa. The three medicinal species of the *Echinacea* genus were distinguished by RAPD analysis. Genetic distance analysis has indicated a high degree of difference among the three species with a relative lower difference between *E. angustifolia* and *E. pallida* [5]. SCAR (Sequence Characterized Amplified Region) markers are potential tools for authentication of herbal drugs. Adinolfi., *et al.* [6] developed a SCAR marker to differentiate *Echinacea purpurea* from *E. angustifolia* and *E. pallida.*

Recent advances in genomics involve gas and high performance liquid chromatography, mass spectrometry and data mining for high-throughput metabolic fingerprinting. A high-performance liquid chromatography method, was optimized, and validated for the detection and quantification of the major phenolic compounds: cichoric acid, chlorogenic acid, caftaric acid, cynarin, and echinacoside, in root and aerial parts of dried *E. angustifolia, E. pallida,* and *E. purpurea* [7]. Comparative metabolomics approach coupled with cell- and gene-based assays was employed for species classification and anti-inflammatory bioactivity validation of *Echinacea* plants [8].

For production of high-quality *Echinacea* for medicinal plant preparations, it is necessary to eliminate the chemical variability, eliminate abiotic and biotic contamination, breed elite plant genotypes and optimize the growing systems. Transformation systems based on *Agrobacterium tumefaciens* are well established for *Echinacea* species [9]. Cloning the genes controlling the production of medicinal compounds will yield commercially useful transgenic plants capable of producing important secondary metabolites.

Peppermint *(Mentha piperita L)*

Mentha is a genus of aromatic perennial herbs belonging to the family Lamiaceae. It is distributed mostly in the temperate and sub-temperate regions of the world. Several *Mentha* species are considered industrial crops as they are a source of essential oils enriched in certain monoterpenes, widely used in food, flavor, cos-

metic and pharmaceutical industries. *Mentha* has a large number of species that differ widely in their characteristics and polyploidy level. It is known to comprise about forty recognizable species. The similarity and diversity based on RAPD profiles of released cultivars of different peppermint species including *Mentha piperita* have been described [10]. Additionally, nuclear DNA (ITS), chloroplast DNA (non-coding regions trnL intron, intergenic spacers trnL-trnF, and psbA-trnH), and AFLP and ISSR, markers were used to reconstruct the phylogeny of mints related to M. piperita [11]. Recently, a total of 1316 ESTs was used to develop a set of SSR markers which can be used for diversity analysis among species and accessions of Mentha [12].

In Mentha species, essential oil biosynthesis and storage is restricted to the peltate glandular trichomes (oil glands). A functional genomics approach towards the characterization of genes involved in essential oil formation in peppermint has been employed. Sequence information from 1,316 randomly selected cDNA clones, or expressed sequence tags (ESTs), from a peppermint (*Mentha piperita*) oil gland secretory cell cDNA library has been obtained [13]. Furthermore, a systems biology approach was employed to identify the biochemical mechanisms regulating monoterpenoid essential oil composition in peppermint [14].

Genetic engineering to up-regulate a flux-limiting step and down-regulate a side route reaction has led to improvement in the composition and yield of peppermint oil. Practical levels of field resistance to glufosinate in peppermint have been achieved and attempts to enhance yield-limiting pathway steps have also been productive [15,16]. Transgenic peppermint plants overexpressing the gene coding for (−)-limonene 3-hydroxylase (L3H) did not accumulate increased levels of the recombinant protein, and the composition and yield of the essential oils were the same as in wild-type controls; however, co-suppression of the L3H gene resulted in a vastly increased accumulation of the intermediate (−)-limonene, without notable effects on oil yield (elite transgenic line designed $L3H_20$) [17].

How to design efficient breeding strategies

Characterization of genetic diversity within these collections is a necessary prerequisite to their efficient use. Recent technological advances in the areas of DNA sequencing and genotyping are serving to redefine the scope of germplasm characterization [18,19]. Importantly, in the near future, advances in crop improvement will be possible by combining genomic tools with rationale selection of germplasm and precise phenotyping for traits of interest - an approach termed genomics-enabled molecular breeding [20].

By bringing specialty crops into cultivation, traditional and biotechnological plant-breeding techniques can be applied at the genetic level to improve yield and uniformity of the active compounds, and to modify potency or toxicity. Selection assisted by genetic markers is an extension of traditional crop breeding, which has been used extensively in food crop improvement. Again, it is a way to recognize desirable genotypes at an early stage to speed up the selection process. However, it is imperative to carefully prioritize the traits for marker development as well as simplifying and optimizing methods to reduce marker genotyping costs. The target compounds are almost invariably secondary metabolites, which, for the plant, frequently serve as adaptations to fluctuating temperature and light conditions (e.g. antioxidants), stress (e.g. proline), infection (e.g. flavanoids) or herbivory (e.g. alkaloids). For example, shade-grown *Mentha piperata* has a lower essential oil content (1.09% v 1.43%) and lower menthol content within the oil (57.5%v 61.8%) compared with light-grown *Mentha piperata* [21].

Data mining of genomics database could be an effective strategy to increase the collection of DNA markers in specialty crops. The model plant sunflower is in the same plant family as coneflower (*Echinacea angustifolia* DC). The rapid increase in detailed genomics resources for sunflower (*Helianthus annus*) opens up opportunities for the improvement of these medicinal species via comparative genetics. To date, however, there have been only few reports of molecular marker-based approaches to medicinal plant improvement [22]. *Echinacea* secondary metabolites could be a source of new drugs for pharmaceutical industry. Cloning the genes controlling the production of medicinal compounds and more efficient and robust transformation systems will yield commercially useful transgenic roots and plants capable of producing important secondary metabolites. Wang and To [9] developed transgenic *Echinacea* plants overexpressing Petunia chalcone synthase gene that can be used as a model system for studying the accumulation of plant secondary metabolites.

Direct manipulation of DNA sequences to alter gene expression in medicinal plants is an area that is ripe for expansion. Genetic transformation systems of high efficiency are available for peppermint so improvement of this crop through biotechnology is very feasible. Strategies might involve transformation with genes for herbicide, disease, insect, cold and drought resistance and bioengineering of the biosynthetic pathways to modify essential oil production. Modulation of essential oil biosynthesis can be approached by metabolic engineering or through regulating trichome differentiation and development. Promoters that condition expression in response to inducers, in spatial and temporal contexts, and as a function of development are likely needed to realize the full potential of biotechnology [23].

Conflict of Interest

The author declares that there are no financial interest and conflict of interest.

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Volume 1 Issue 4 October 2017