Barley

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Contributor: Paolo Pesaresi

Barley (Hordeum vulgare) has been widely used as a model crop for studying molecular and physiological processes such as chloroplast development and photosynthesis. During the second half of the 20th century, mutants such as albostrians led to the discovery of the nuclear-encoded, plastid-localized RNA polymerase and the retrograde (chloroplast-to-nucleus) signalling communication pathway, while chlorina-f2 and xantha mutants helped to shed light on the chlorophyll biosynthetic pathway, on the light-harvesting proteins and on the organization of the photosynthetic apparatus. However, during the last 30 years, a large fraction of chloroplast research has switched to the more “user-friendly” model species Arabidopsis thaliana, the first plant species whose genome was sequenced and published at the end of 2000. Despite its many advantages, Arabidopsis has some important limitations compared to barley, including the lack of a real canopy and the absence of the proplastid-to-chloroplast developmental gradient across the leaf blade. These features, together with the availability of large collections of natural genetic diversity and mutant populations for barley, a complete genome assembly and protocols for genetic transformation and gene editing, have relaunched barley as an ideal model species for chloroplast research. In this review, we provide an update on the genomics tools now available for barley, and review the biotechnological strategies reported to increase photosynthesis eciency in model species, which deserve to be validated in barley.

Keywords: barley ; functional genomics ; photosynthesis improvement ; chloroplast biogenesis ; chloroplast-nucleus communication ; molecular breeding

1. Barley, the Crop and the Model Species

Barley (*Hordeum vulgare*) is a self-pollinating monocotyledonous plant species that belongs to the *Poaceae*, a grass family that includes several major crops exploited in modern agriculture. Its domestication dates back to 10,000 BC, took place in the Fertile Crescent and began with the wild species *Hordeum vulgare* ssp. *spontaneum* [1]. Barley ranks fourth in terms of annual grain tonnage after maize, wheat and rice, with a worldwide production level (2018/2019) of 141 million tons. The primary role of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) is as a source of animal feed (about 75% of the global production), with subsidiary uses in alcoholic and non-alcoholic beverages (20%), and in human nutrition (5%)—partly due to its high content of beta-glucan, a beneficial fibre that can reduce levels of cholesterol in the blood. During the 20th century, barley was widely exploited as a model species for crop studies. As a self-pollinating species with a diploid (2n) genome and a haploid complement of only seven chromosomes, barley proved to be an excellent model organism for both basic and applied research. Furthermore, due to the fact that wild barley (*Hordeum vulgare* ssp. *spontaneum*) can grow in a wide range of environments and climates, from the Arctic Circle to the equatorial highlands, barley is cultivated more widely than any other major crop. This resilience relies on a wealth of natural genetic diversity which enables the plant to adapt effectively to various environmental challenges such as cold temperatures, drought, alkalinity and salinity, and makes it a perfect model species for investigating crop adaptation to abiotic stresses [2].

2. A Brief History of Genome Manipulation in Barley

Hordeum vulgare was one of the very first crops used in cereal improvement programs based on different induced mutation strategies. In 1930, Stadler studied the mutagenic effects of different types of radiation on maize and barley, describing chlorophyll-deficient and virescent phenotypes in seedlings [3]. In 1938, Nilsson-Ehle and Gustafsson tested X-rays and UV light on the barley cultivar (cv.) ‘Gull’ and isolated several mutants, which were named *albina*, *xantha*, *alboviridis*, *viridis, tigrina, striata* and *maculata*, categorizing them by their carotenoid and chlorophyll contents and distribution within the leaf blade [4]. The characteristics of several mutated lines were recognized as being very valuable for potential use in agriculture, since they exhibited alterations in grain yield, straw stiffness, straw

length and tillering capacity, as well as changes in spike firmness, kernel maturation and pigmentation [5]. Later on, two varieties of barley 'Trebi' and 'Moister' were exposed to the radiation generated by the first aerial atomic explosion at Bikini atoll in 1946 [6]. Meanwhile, Gustafsson and Mackey applied mustard gas to barley to observe the effect of chemical mutagenesis [7], whereas Ehrenberg and collaborators tested various mutagenic compounds on barley and evaluated their impact on chlorophyll accumulation [8]. After these pioneering experiments, a broad range of chemical and physical mutagens were tested systematically. During this phase, alkylating agents able to generate G/C to A/T transitions in DNA, such as EMS (Ethyl Methane Sulfonate), ENU (N-nitroso-N-ethylurea), MNU (N-nitroso-N-methylurea), DES (diEthyl Sulfate) and sodium azide (NaN_3), were widely used for the mutagenesis of barley. The first chemically induced barley variety, 'Luther', was released in the US in 1966. 'Luther' was obtained by exposing the variety 'Alpine' to DES. In 1965, in Czechoslovakia, the variety 'Diamant' was obtained after gamma-ray irradiation. This new variety was ~ 15 cm shorter than the parental 'Valticky' and displayed an increase in grain yield amounting to about 12% [9]. At around the same time, in the UK, 'Golden Promise' was registered. This semi-dwarf cultivar originated from exposure of the salt-sensitive variety 'Maythorpe' to gamma rays [10]. The generation of 'Golden Promise' represented an important step towards the development of tissue culture and barley transformation techniques (see below).

3. Early Studies and Milestones in Understanding of Chloroplast Biogenesis and Physiology in Barley

Genetic studies of barley have not been restricted to breeding programs. The plant has also been used as a model species to dissect the molecular mechanisms that underlie plant development and physiology and, for a large part of the 20th century, it served as a major experimental system for the investigation of chloroplast biogenesis and photosynthesis. In particular, several studies during the 1970s characterized different aspects of plastid structure and development, such as plastid growth, replication and differentiation. Dark-grown barley seedlings were used to determine the protochlorophyll content and structure of the etioplasts. Exposure to different light conditions allowed chloroplast development to be characterised from both structural and biochemical points of view [11,12,13,14].

The organization of chloroplast membranes was analysed in chloroplast preparations solubilised with digitonin and fractionated by electrophoresis, proving the existence of distinct sets of membranes [15]. The functionality and structural organization of thylakoids were also studied in barley mutants altered in chlorophyll biosynthesis [16] and revealed the impact of such changes on thylakoid membrane organization. For instance, the *chlorina-f2* mutant, which is impaired in chlorophyll b accumulation, led to the discovery of light-harvesting chlorophyll-binding proteins [17,18,19,20]. *Chlorina-f2* was also used to assess the impact of protein-chlorophyll complexes on the ultrastructure of thylakoid membranes, shedding light on the organisation of the photosynthetic apparatus [17,21]. In addition, the *tigrina-d* mutant [22], originally suggested to be involved in the early steps of tetrapyrrole biosynthesis prior to ALA formation, was recently identified as the barley orthologue [23] of the *FLU* gene of *Arabidopsis thaliana*, a nuclear-encoded, plastid-localized protein that plays a key role in the negative-feedback control of chlorophyll biosynthesis, with an essential role during the dark-to-light switch [24]. Moreover, the barley *xantha* mutants helped to elucidate key steps in chlorophyll biosynthesis [25]: *xantha-l* was shown to code for a mutated form of Mg-protoporphyrin IX monomethyl ester cyclase, while *xantha-f*, *-g*, and *-h* carry genetic lesions at three distinct loci encoding the three Mg-chelatase subunits [26,27].

From a physiological point of view, Smith et al. [28] documented changes in chloroplast activity during de-etiolation of barley seedlings by measuring the Hill reaction in relation to chlorophyll accumulation. The correlation between plastid ultrastructure, chlorophyll synthesis and development of photosynthetic activity was also evaluated by measuring O_2 evolution [29].

Besides the characterization of the photosynthetic apparatus, barley played an important part in the dissection of the chloroplast's gene expression machinery. Indeed, evidence for a fully nuclear-encoded transcriptional activity in plastids, later named the Nuclear-Encoded RNA Polymerase (NEP; [30]), was first reported in barley, based on analysis of the *albostrians* mutant. In particular, the synthesis of RNA was reported in the white sectors of *albostrians* leaves, which harbor plastids that are devoid of ribosomes. These data provided initial evidence for the existence of a nuclear-encoded and plastid-localized RNA polymerase [31]. In addition, ribosome-free plastids of *albostrians* were helpful in distinguishing between the set of plastid genes preferentially transcribed by NEP, such as *rRNA*, *rpo* and *rps15*, and the set transcribed by the Plastid-Encoded RNA polymerase (PEP), which is enriched in photosynthesis-related genes such as *psbA*, *rbcL*, *atpI-H* [31,32]. Furthermore, the barley *albostrians* mutant was essential to the initial detection of communication between organellar and nuclear genomes. By analyzing *albostrians*, which is characterized by reduced amounts and/or activities of nucleus-encoded chloroplast proteins including the small subunit of ribulose-1,5-bisphosphate carboxylase⁄oxygenase (Rubisco), ferredoxin NADP+ reductase, and enzymes of the Calvin cycle, Börner provided the

first evidence for plastid signals that control nuclear gene expression, leading to the discovery of chloroplast-to-nucleus retrograde communication [33,34,35].

4. Influences

Its genetic diversity and the availability of a large collection of molecular tools make barley an ideal model crop for functional genomics studies related to chloroplast biogenesis and retrograde communication. Such studies will reveal to what extent retrograde signalling mechanisms are conserved between Arabidopsis and barley, and permit us to learn more about aspects of chloroplast biogenesis that are specific to monocots. The recent identification of the genetic factor responsible for the *albostrians* phenotype demonstrates that this type of analysis can now be effectively conducted in barley. The fact that the gene concerned, *HvCMF7*, encodes a protein that is apparently located exclusively in plastids highlights the need for systematic investigation of barley mutants with defects in chloroplast biogenesis. Furthermore, novel approaches to the screening of barley mutant populations are required to elucidate the molecular details of the chloroplast-to-nucleus communication. The genes and allelic variants identified in future studies could have an important impact in breeding programs, since retrograde communication controls the leaf life cycle.

Barley can also make a significant contribution to the testing of novel biotechnological strategies for improving photosynthesis, and the validation of their effects on biomass accumulation and grain yield. In recent decades, our knowledge of the photosynthetic process has increased substantially, and improvements in its efficiency have been demonstrated in different model species. The high level of conservation of the photosynthetic process strongly argues that similar enhancements can be achieved in barley. Thanks to the high content of sugars in the straw, barley could be transformed into a dual-purpose crop suitable for the production of biofuel from the straw, and food, feed and spirits from the grain. Furthermore, the use of barley varieties characterised by high photosynthetic efficiency and reduced antenna size of photosystems is a promising strategy for boosting productivity and water use efficiency, while increasing land–surface reflectivity to offset greenhouse gas warming. In light of the foreseeable rise in the demand for food by the middle of this century, and the fact that the development and commercialization of a new plant variety with improved quality takes 10 to 15 years, concerted efforts to increase agricultural yields through manipulation of photosynthesis must be initiated immediately. The “redesign” of photosynthesis must represent one of the main pillars of the next “Green Revolution”.