Photoreceptor Biotechnology in the Aid of Agronomical Advancement

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Abstract—*Photoreceptors exert profound influence on various aspects of plant biology starting from seed germination, photosynthetic resource allocation, leaf formation to nitrogen fixation and circardian clock. This multi-faceted grip of photoreceptors on crop yield makes them an appealing target for biotechnological approaches. Manipulating or smart breeding of the genes involved in photoreceptor expression or their signal transduction components could be used to modify various aspects of plant development and metabolism. Evidence of the capability of phytochrome photoreceptors to affect plant growth and development is evident by the severe pleiotropic phenotypes of mutants in multiple photoreceptor genes. Applications of plant photoreceptors are limited not only to the engineering of plant development and metabolism only. Knowledge of the structure of phytochromes can make them impact molecular biotechnology. The unique biochemical properties of these phytochromes, project them as attractive candidates for molecular biotechnology with wide-ranging applications. Now it is possible to optimize light-driven gene control systems or phytofluors by intelligent domain-swap or sitedirected mutagenesis of key residues involved in photoconversion. Such ability to make intelligent, evidence-driven modifications may thereby pave the way to photoreceptor scientists to move out of the "dark ages" and into a new era of advanced protein design.*

1. INTRODUCTION

Plant photoreceptors play a very crucial role in influencing many agriculturally relevant traits. All aspects of plant metabolism like the regulation of enzymes and other proteins mediating photosynthesis, growth and development including seed germination, circardian rhythm, seeding architecture dormancy, leaf size and shape, flowering time etc are affected by photoreceptors with varying extent. Because of the importance of many photoreceptor-controlled processes in determining the yield of crop, there has been a lot of emerging interest in using biotechnological approaches for crop improvement these days.The major three families of plant photoreceptors are- the red (R) and far-red (FR) light sensing phytochromes, the blue / UVA (B) sensing cryptochromes (see Batschauer et al.) and the B sensing phototropins. As the phytochromes have been studied for a long time and the over-expression constructs of the genes are available, they have attracted the maximum attention. Phytochrome overexpressors are much desirable from the perspective of yield and harvesting time. The action of the blue light (B) sensing cryptochromes are similar to that of phytochromes. The phototropins are associated with the blue light induced phototropic responses(Kagawa, 2003), chloroplast positioning, leaf expansion and stomatal opening. Altering the expression of the phototropins or their signaling partners can modify these responses. Mutations in multiple photoreceptors, or mutants in the synthesis of the phytochrome chromophore show severe pleiotropic phenotypes (Hudson, 2000). Biochemical properties unique to the plant photoreceptors make them attractive candidates for wide range of biotechnological applications. The phytochrome holoprotein exixts in two photoconvertable states - R and FR. This bistable property of is exploited as a molecular switch.

2. APPROACHES TO MODIFY THE PHOTOMORPHOGENIC RESPONSES IN CROP PLANTS

2.1 Dwarfing of plants

Dwarfing of plants increases yield by partitioning more photosynthate to the grain at the expense of reducing the resources allocated to structural growth(Salamini, 2003). It also renders more resistance to mechanical flattening by rain or wind. Dwarf crops usually carry mutant alleles affecting gibberelin pathways (Peng et al., 1999). Creation of such mutants alter growth and development as well as reduce photosynthetic capacity. Photoreceptor over-expression creates dwarf plants which are not compromised in any aspect of development. This can serve as potential tool in agronomic applications.

2.2 The shade-avoidance response

The response of plants to vegetation shade is termed the "shade avoidance syndrome" (Smith, 1995). This is mostly mediated by perception of light spectral quality. Both resource partitioning and growth patterns are significantly influenced by this syndrome (Smith, 1995, Smith, 1981, 1983, Robson et al., 1996, Gilbert et al., 2001). Even though hade avoiding plants exhibit rapid elongation growth and accelerated reproduction, there is a decrease in the number of embryos and less photosynthate to storage organs like tubers. Thereby causing yield loss and undesirable morphology. Modification of shade avoidance has a crucial role to play in crop improvement. Plants are sensitive to crowding because they respond to the spectral quality of vegetation shade via the phytochrome family of photoreceptors. Plants overexpressing phytochromes reduce the extent to which the shade-avoidance syndrome influences plant morphology and development. PhyB overexpression successfully modifies shade avoidance characteristics and helps to achieve increased yield in field crops. Overexpression of phytochrome A (phyA) extends the far-red high irradiance response which is antagonizes shade-avoidance syndrome(Casal et al., 1997, McCormac et al., 1992).

2.3 Control of gene expression and shade avoidance

Understanding of the downstream mechanisms involved in the shade- avoidance has the potential to target specific responses without affecting the other features. As for example in Arabidopsis, mutation in LONG HYPOCOTYL IN FAR-RED1 (HFR1) gene showed increased shade avoidance responses. Thus HFR1 negatively regulates shade avoidance so as to prevent death of seedlings.

2.4 Alteration of the timing of flowering

Manipulating the time of crop flowering has tremendous potential for crop improvement. Phytochrome over-expression delays flowering (Robson and Smith, 1997) whereas loss-of-function mutants generally flowers early(Childs et al., 1997), or are insensitive to photoperiodism. Cryptochrome gene mutants reduce the sensitivity to photoperiod, but have the reverse effect in Arabidopsis. In contrast, cryptochrome mutants of pea flowers early(Platten et al., 2005). The variation in the photoperiodic responses in addition to the altered photoreceptor levels may lead to pleiotropic effects thereby making photoreceptor modification a blunt tool to increase crop yield.

2.5 Taxonomic differences and similarities in higher plants

The taxonomic differences in photoreceptors and photomorphogenesis between plant species complicates the use of photoreceptor biotechnology. Photoreceptor systems have themselves experienced independent evolution within the angiosperms. Monocots have three phytochromes genes, *PHYA*, *PHYB* and *PHYC* whereas in dicots there are multiple types of PHYB. In addition the response exerted by each of them varies between monocots and dicots. These diversities complicate designing of experiments for phytochrome response modification. Based on transgenic experiments, signaling mechanisms of photomorphogenesis has emerged. Exploiting them like introducing an *Arabidopsis PHYA* gene into rice causes increased dwarfing and thus is prospective of enhancing the yield.

3. MODIFICATION OF PHOTOMORPHOGENESIS USING GENETIC TRANSFORMATION

3.1 Plants transgenic for phytochromes

Expression cassettes designed for the over-expression of phytochrome genes have been used to modify a large number of plant species which show substantial dwarfing and increased yield (Figure-1). The best example is potato where extra copies of the potato *PHYA* and Arabidopsis *PHYB* genes have been introduced into potato under the control of the 35S promoter.PHYA (Heyer et al., 1995, Thiele et al., 1999) leads to dwarfing and PHYB substantial increase in tuberization (Theile et al., 1999), consistent with reduced shade-avoidance phenotype. In *S. tuberosum ssp. Andigena* tuberization is dependent on short day conditions. PHYB antisense ablation allows tubers to form under long day conditions thereby modifying yield characteristics with the aid of photoreceptor biotechnology.

3.2 Modification of other photoreceptors

Lesser availability of the cryptochrome genes has been an issue for concentrating more on phytochromes, however recent studies on tomato where cryptochrome is over-expressed has shifted the focus to the later. The fruit anti-oxidant content increases which is likely to modify plant development.

3.3 Overexpression of signaling components

Mutation and over-expression of signaling components has the potential to target specific aspects of photomorphogenesis and thus biotechnology application. As for example NDPK2 knockouts affect seedling hook opening (Choi et al., 1999).

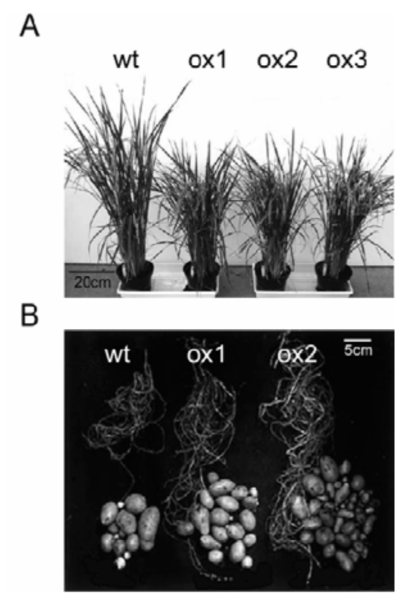

Fig. 1: Dwarfing and yield increase using phytochrome overexpression.

A. Phenotype of greenhouse-grown rice plants overexpressing Arabidopsis phyA. The transgenic lines (ox1, ox2 and ox3) are all substantially shorter than wild type plants, and have shorter tiller internodes. The lines also showed a yield increase of 21%, 6% and 11% respectively. Adapted from Garg et al. (2005). Copyright Springer Publishing, 2005.

B. Increase in number and yield of tubers in potato plants overexpressing phytochrome B. The wild type (WT) and phyB-overexpressing transgenics (ox1 and ox2) were grown to harvest under greenhouse conditions. The ox2 line overexpresses phyB holoprotein at higher levels than ox1, consequently the tuber number increase is proportional to the level of phyB. Adapted from Thiele et al. (1999). Copyright American Society of Plant Biologists, 1999.

4. MODIFICATION OF PHOTOMORPHOGENESIS BY UTILIZING GENETIC DIVERSITY

4.1 Natural variation in photomorphogenesis

Undertstanding the effect of photoreceptors and photomorphogenic alleles in evolution and natural selection is making rapid progress in recent years. Both increased and decreased expression of phytochromes substantially reduce the fitness of plants, which may not be detrimental but definitely will produce traits for sub-optimal yields. Especially in shade-avoidance syndrome, variants displaying reduced shade avoidance will appear unhealthy (Schmitt et al).

4.2 Photoreceptors and photomorphogenic genes as targets for selection in crops

Genetic diversity in photomorphogenic pathways forms an untapped resource for crop yield improvement without the necessity of chemical applications or transgenes. Most of the morphological traits of modern crop plants increase tolerance to higher planting densities. Keeping in mind the effect they have on the morphology and resource partitioning in densely grown crops, photoreceptor genes determine the yield (Robson, 1996; Robson and Smith, 1997), especially when shade-avoidance is a strong factor. Hence indirect selections have been exerted on photomorphogenic traits during the breeding of modern crops. As for example, in maize most of the significant increases in yield is produced by higher tolerance for crowding (Duvick, 1997) and not by an increase in yield on a per-plant basis. Since photosensitivity can impart a negative effect, breeders have altered their selection.

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5. FUTURE DIRECTIONS IN PHOTORECEPTOR BIOTECHNOLOGY

With the substantial advancement in genomics, details of photomorphogenesis and genome projects of crops like maize has been nearing completion. This has opened up newer windows to generate tools for the utilization of photoreceptors or their signaling pathways for targeted dwarfing alteration of shade avoidance or manipulating other traits. Resquencing studies across large germ plasm collections will result in generation of large databases of genetic diversity increasing knowledge of polymorphisms among crop cultivars. Such data will lead to the discovery of photomorphogenic alleles which are likely to become the focus of targeted “smart breeding” approaches. Thereby making photoreceptors and photomorphogenesis very crucial to the eyes of crop breeders.

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