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Development of Genetically Engineered Plant Photoreceptors for Generating Crop Plants with Novel Agronomic Traits

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Abstract

Regulatory mechanism exerted by photoreceptors makes them potential targets for biotechnology and genetic engineering approaches. Plant photoreceptors influence crop improvement by regulating plant metabolic processes including seed germination, circadian time control, seedling de-etiolation, photosynthetic resource allocation, chloroplast development and positioning, flowering time, grain filling and dormancy. Genetic engineering strategies involving photoreceptor genes or even the components of their signal transduction pathway are helpful in modifying various aspects in development and metabolism of plants. Out of various plant photoreceptors available, Phytochromes are potential tools in biotechnology studies as their over expression would alter various plant development processes including dwarf induction, enhancing shade of responses and delayed flowering. Over expressing *Arabidopsis* PhyA gene in aromatic rice varieties make plants dwarf with increased yield. Dwarfing can also be achieved with Cryptochrome genes. These genes are also useful in enhancing antioxidant content of fruits. Plants that are ready to get transformed are modified using expression cassettes which over express Phytochrome genes. The fitness of plants is highly influenced by reduced as well as increased Phytochrome expression. Studies have been increased with Phytochrome mutants in understanding its potential role in crop development. Plant photoreceptors can be used not just for genetic engineering that would enhance the process of development and metabolism. The property of Holoprotein to shift between R and FR states makes Phytochromes a potential molecular switch that could be used for the regulation of gene expression. Considering various applications of photoreceptors, concrete knowledge on the structure of Phytochrome would significantly influence Phytochrome based molecular biotechnology. The potentials of photoreceptors in modifying various developmental processes might lead scientists to step forward near the era of designing protein in advanced manner.

Keywords: Photoreceptors, Arabidopsis Phy A, Gene expression, Cryptochrome, Regulation

1. Introduction

Plant photoreceptors are responsible for controlling different aspects involved in plant development. This is proved by severe pleiotropic phenotypes including mutations in multiple photoreceptors or even mutations occurring in Phytochrome chromophore biosynthesis (Hudson, 2000). Different plants will have different time for processes including metabolism, growth and development. Photoreceptors influence most agriculturally important traits in a strong manner. Such traits are germination of seeds, circadian timing, architecture of seedling, bud dormancy, shape and size of leaves, length and curvature of shoot, development and positioning of chloroplasts, time taken for flowering, grain filling as well as dormancy including bud dormancy. Most of metabolic processes are mediated by light. Some processes like nitrogen fixation and gas exchange are mediated both by light as well as circadian clock. Regulations of genes that are involved in such metabolic processes are highly regulated by photoreceptors. Genoud et al found that the defense mechanism in plants is highly influenced by these photoreceptors (Genoud et al, 2002). As many processes involved in plant development are highly regulated by photoreceptors, significant interest has been raised on these photoreceptors to understand these processes better.

The controlling mechanism exerted by these photoreceptors is the promising target in developing biotechnology approaches. Genetic engineering strategies involving photoreceptor genes or even the components of their signal transduction pathway are helpful in modifying various aspects in development and metabolism of plants. Plant photoreceptors like phytochromes possess certain biochemical properties that enable them to be exploited as potential tools for molecular biotechnology. Bi-stable ability of phytochromes existing in two states responding to pulses of Red (R) and Far Red (FR) makes it possible to be used as molecular switch. Among three families of plant photoreceptors identified till date, phytochromes are known as potential tools in biotechnology studies. This is because; a) Phytochromes are well known photoreceptors than others, b) they can be over expressed by constructing cassettes, etc. Over expression of phytochromes are successful in obtaining maximum yield with novel traits. Modifying responses of photoreceptors against light by altering expression pattern of phototrophin molecules and also their signaling components were potentially mentioned in few scholarly articles worked on biotechnology applications of these molecules.

2. Modification of Different Photomorphogenic Responses in Crop Plants using Phytochromes

2.1 Photoreceptors can induce dwarfing

Dwarfing method would increase the yield and in some cases, certain novel traits can be highlighted in crop plants when resources available for structural are reduced, which can be used to achieve the same. This process also enables crops to develop resistance against mechanical flattening induced by wind or rain in certain cases. Most of dwarf crops are found to carry mutations in alleles that are involved in Gibberellin pathways (Peng et al, 1999). Despite the fact that there are various ways in which you can achieve dwarfing, over-expression of photoreceptor is found to be the potential tool as others include technical difficulties. The best example is that over-expression of *Arabidopsis* PhyA gene in aromatic rice varieties induced dwarfing (Garg et al, 2005). Over-expression of monocot Phy A gene in oat or rice showed strong dwarfing resulting in increased crop.

2.2 Shade avoidance Response

Depending on the quality of light spectrum, plant responses to vegetation shade are well catalyzed which are called “shade avoidance syndrome”. This syndrome regulates partitioning of resources that are required for growth and thereby controlling growth patterns (Smith, 1995). Plants that avoid shade are found to have rapid elongated growth with increased reproduction by compromising expansion of leaf and production of photosynthetic pigments. When the number of embryos developed in such plants is reduced, it increases the yield of seeds. Modifying shade avoidance response in crops would be helpful in yielding high quality crops.

Plants are capable of differentiating light quality absorbed through solar irradiation taken by chlorophyll even in the condition where the total photosynthetically active radiation (PAR) is maximum. This is confirmed through the relation where the ratio of red to Far red light (R/FR) and also the state of equilibrium happen between the active form (Pfr) as well as inactive form (Pr) of phytochromes, which is found to be proportional to vegetation density (Smith, 1995). This proportionality is found to exist when FR quantity is more than R. This confirms that Phytochrome family helps plants in responding towards spectral quality of vegetation shade. Phytochrome gene Phy B is found to have promising role in shade avoidance (Quail, 1994). Over-expression of this gene Phy B yield more crops with modified share avoidance traits. Similarly, another Phytochrome gene called Phy A is found to show antagonizing shade avoidance responses in plants when they are over-expressed (McCormac et al, 1992). This gene has property to respond more to FR than R (Casal et al, 1997).

2.2.1 *Phytochromes control gene expression in shade avoidance responses*

Complete knowledge of mechanisms by which shade avoidance responses happen is necessary for finding the potential targets to induce modification. Post finding targets, modification of shade avoidance response patterns in plants are possible. For instance, we know that modifying resource allocation responding to canopy shade with no effects on flowering time or even harvesting time, the actual role of Phytochrome in such modification is not still explained clearly. In *Arabidopsis,* there are three factors that help in mediating shade avoidance pathways that can serve as potential targets which include PIL1, ATHB-2 and HFR1.

2.3 Changes in flowering time

Changing the flowering time would be helpful in increasing crop yield. Similarly, for other traits including fruit ripening time, tuberization time, grain filing time, etc can also be altered using phytochromes. By over-expressing phytochromes, the flowering time can be delayed (Robson and Smith, 1997). When Phytochrome genes responsible in delaying flowering time are lost in any plant, then it results in early flowering like in case of Ma3R allele of Sorghum (Childs et al, 1997). Using antisense therapy by introducing phyB transcript in potato resulted in removal of photoperiod requirement in tuberization process (Jackson et al, 1996). In *Arabidopsis*, mutant form of cryptochrome genes resulted in reduced sensitivity against photoperiod inducing delayed flowering under inductive conditions. These details confirm that photoreceptors can be used as molecular tool in modifying flower time and harvesting time without disturbing yield.

2.4 Photoreceptors induce taxonomic differences and similarities

As there are more taxonomic differences and similarities between plants are observed, the use of photoreceptors in modifying growth and development is highly restricted. Additionally, photoreceptor systems have been found to undergone independent evolution inside Angiosperm systems, especially phytochromes (Matthews, 2005). In Rice and other monocots, there are about three major phytochrome genes are present including PhyA, PhyB and PhyC. In dicots, two phytochrome genes such as Phy A and PhyC present in monocots are available. However, Phy B version of Phytochrome gene in monocots is available in Dicots as mutated version, observed in *Arabidopsis*. These are often named as B1, B2, etc. This diversity of type B phytochrome gene is responsible for increasing selection pressure on phytochrome genes resulting in induction of shade avoidance responses.

2.5 Modification to other traits

Over-expression of phytochrome gene PhyB results in increased tuberization process. This shows that plants growth in regulated environment can yield greater tubers. Antisense technology paves way to identify the potential difference between *S. tuberosum* and *S. andigena* in their tuberization processes induced by PhyB phytochrome gene. Patented form of phytochrome constructs are available specifically for dwarfing, for modifying shade avoidance responses and also for changing flowering time or harvesting time.

3. Modifying Photomorphogenesis through Genetic Transformation of Phytochromes

3.1 Plants containing different forms of phytochromes

Introducing expression cassettes used for over-expressing phytochrome genes is helpful in modifying transformation process in different varieties of plant species. With successful knowledge about transformation of phytochromes in lower plants including *Arabidopsis*, it is now possible to include transformation process in rice to get maximum yield (Clough et al, 1995). Over-expression of phytochrome gene in potato plant potentially increased yield. Under the control of strong 35S promoter, few additional copies of PhyA potato genes as well as PhyB *Arabidopsis* genes are found to have introduced into potato plants.

3.2 Modification observed by photoreceptors other than phytochromes

In *Arabidopsis* and Rice varieties, for understanding the role of cryptochromes and phototrophins, loss of function mutations are highly helpful. Most of dwarfing studies are done with phytochromes while cryptochromes can also induce dwarfing. This is because of unavailability of cryptochromes for longer time. Recent studies suggested that over-expression of cryptochrome in Tomato plant (Giliberto et al, 2005) shown to increase fruit antioxidant content with fewer negative impact. This makes the scientist Giliberto to rely on cryptochrome for modifying growth and development pattern of plants.

3.5 Signaling components can be over-expressed to yield novel traits

Several restrictions arise to carry out modification in morphology through mutation as well as over-expression of signaling molecules (Quail, 2004). For instance, NDPK2 knockouts are found to affect opening of seedling hook without affecting the elongation of hypocotyls (Choi et al, 1999). As it is clear that only one trait at a same time can be concentrated for modification without affecting others is the great aspect which makes these photoreceptors ideal for biotechnology application.

4. Modifying Photomorphogenesis through Genetic Diversity

4.1 Changes in Photomorphogenesis occurring naturally

Recent studies focused on understanding the potential role of photoreceptors as well as photomorphogenic alleles involved in variation and evolution that happen naturally. The fitness of plants against canopy environments are highly influenced by both reduced as well as increased expression of phytochromes (Schmitt et al, 1995). Irrespective of whether it a wild plant or a breed, the selection pressure on these genes is found to happen. Using *Arabidopsis*, description of molecular basis of variable responses can be achieved. Photobiologists are surprised to observe naturally occurring mutant varieties of photoreceptors within the population of *Arabidopsis*. For instance, natural mutation is found to happen in phytochrome gene PhyD (Aukerman et al, 1997) that results in WS accession in *Arabidopsis*. The above result is confirmed again with the discovery of mutated Phy A gene occurring naturally within the plant species with greatly reduced FR sensitivity, which results in Lm-2 accession (Maloof et al, 2001). These show that naturally occurring mutations in photoreceptor sequences can greatly influence almost all agronomically potential traits including reproduction time or harvest time. Therefore, studying such natural variations through genomic approaches have been started to understand the mechanisms in *Arabidopsis* (Maloof, 2003; Shimizu and Purugganan, 2005). It is also been found that microarray profiling would be helpful in changing the expression pattern of *Arabidopsis* gene is PhyB transcript (Chen et al, 2005). These variations are due to the presence of large degree of diversity in gene sequence especially in promoter region as well as intron regions of Phytochrome gene PhyB.

4.2 Photoreceptors as potential markers for selecting crops

Genetic diversity happen in photomorphogenic pathways found to lie within germ plasm of various crops, which creates the resource for improving crops. While considering the fact that crops whose photomorphogenic systems are under optimum levels with respect to their photomorphogenic responses, it is found that most of morphological traits in modern crop plants can be selected for increasing resistance or tolerance against higher planting densities. When these systems control morphology as well as resource partitioning pattern in grown crops, yield is greatly determined by photoreceptor genes especially at high planting densities in conditions where shade avoidance strongly influence yield determination (Robson, 1996; Robson and Smith, 1997). Additionally, at higher planting densities increase yield by significantly increasing tolerance against drought as well as other stresses (Bruce, 2002). One potential strategy that increases crop yields is by understanding and maximizing light signaling systems which allow these crops to develop tolerance against high density planting (Maddonni et al, 2001; Maddonni et al, 2002).

5. Role of Photoreceptor Biotechnology

5.1 Controlling Gene Expression

In *Arabidopsis*, photoreceptor biotechnology applications are well confirmed by specific bond between PIF3 bHLH transcription factor and the active form of phytochrome Pfr especially in PhyB gene (Ni et al, 1999). Then in any organism, it is found easy for creating a complete light induced signal transduction system. Tightly regulated gene expression system is found in yeast which completely lacks photoreceptor genes (Shimizu-Sato et al, 2002).

Controllable transcription systems are essential tools in both biotechnology as well as biomedical studies. Any of these approaches include the introduction of a small molecular regulator that either enhances or suppresses the activity of genes, thereby affecting mRNA production. One important constraint is that this small molecular regulator once added cannot be retrieved back, which results in either switching on or off gene expression permanently. This constraint was overcome by expression system using LacZ promoter in Yeast developed by Sato and coworkers. The expression system consists of a target gene can be switched on by exposing yeast to red light and can be switched off by exposing yeast to far red light. This expression system influenced by light is created by fusion of PIF3 molecule with the transcriptional activation domain of GAL4 (GAL4D) which results in the formation of chimeric, chromophorylated as well as photo-reversible PhyB-GAL4DNA binding domain protein. This whole system works as light regulated system which is activated while exposing to red light and is suppressed while exposing to far red light. In yeast,

engineering of biosynthetic pathway of a chromophore is possible as it is already done in bacteria (Gambetta and Lagarias, 2001). This type of yeast strains would be helpful for studies that can be conducted in molecular biology laboratories.
Technically, there are no constraints for extending this technology to other organisms including Drosophila or mammalian cell line cultures. Similar to phytochrome, even chromophore biosynthetic genes can be used to make the tool more useful.

5.2 Phytochromes are potential fluorescent probes

Fluorescent proteins are effectively used as fluors for detection. Biliproteins like phycoerythrin have various applications similar to fluorescent proteins. Though phytochrome belongs to biliproteins like phytoerythrin, they are not actually fluorescent proteins. They simply use the energy released on re-emission of photos for their photo-conversion process to yield more Pfr(active form) of phytochrome, which gradually returns back to inactive form Pr when placed in darkness (Murphy and Lagarias, 1997). Discovery of phytofluors is the first remarkable and commercial application of phytochrome biology (Fischer and Lagarias, 2004).

5.3 Additional uses of photoreceptors

As phytochromes have the ability to get inter-converted between two states including red and far red, it can be potentially used as a system to store solar energy. These phytochromes are also potential optical storage devices that can be effectively installed in optical computers (Ni et al, 1999). Phytochrome can be well studied with its crystal structure (Wagner et al, 2005). Considering the properties of photoreceptors with inter-conversion , it is possible to develop light-driven nanomachines in the near future.

Conclusion

Genomics is the vast field which enables us to generate different new tools in modifying photomorphogenesis in plants. As we have more detailed knowledge about photomorphogenesis in most plant species, it is easy to develop interest on photoreceptors and their signaling molecules. With the evidence handy proving the importance of photomorphogenesis in yield determination, alleles involved are becoming the right focus of specific “Smart Breeding” strategies. Considering various applications of photoreceptors, concrete knowledge on the structure of Phytochrome would significantly influence Phytochrome based molecular biotechnology. The potentials of photoreceptors in modifying various developmental processes might lead scientists to step forward near the era of designing protein in advanced manner.

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