5. DISCUSSION

In spite of the identification and characterization of phytochrome several years ago, its molecular action in controlling photomorphogenesis, and the nature of the putative intermediates in signal transmission have still not been fully deciphered. Among the several approaches currently in use, such as development of transgenics, biochemical analysis of pathways, and use of mutants, the analysis of photomorphogenic mutants has been very fruitful to unravel various hitherto unknown facets of photoreceptor action and signal chain intermediates. In the present study, we employed photomorphogenic mutants of tomato to study the role of phytochrome in regulation of processes like enzyme induction, cell differentiation, cotyledon expansion, and Chloroplast development.

5.1 Phytochrome deficiency and cell differentiation

The most interesting aspect of developmental biology is to know how the cell identifies itself, and differentiates accordingly in response to certain endogenous signals and environmental stimuli. In plants, it is known that multiple stimuli can induce similar developmental responses. For example, several photomorphogenic traits are inducible by various environmental stimuli other than light (Chory et al., 1991, 1994). One of the visible signs of photomorphogenesis in dicot seedlings is that while etiolated seedlings have very few hypocotyl hairs, light-grown seedlings have a very high density of hair formation (Mohr, 1972). Similarly,
trichome density on tomato leaves is influenced by both day/night temperatures and photoperiod (Gianfagna et al., 1992).

Molecular genetic analysis has revealed that initiation and expansion of hairs is controlled by no less than 21 genes in *Arabidopsis* of which at least GL1 and TTG gene products are required for initiation. Interestingly, *ttg* mutant which lack hypocotyl hairs have more root hairs than the wild type. Since *au* mutant do possess fewer hairs in dark-grown seedlings, it appear to be defective in controlling the mechanism of hypocotyl hair initiation, and elongation, which appears to be governed by light.

Results from earlier studies clearly demonstrate that the alteration in one developmental pathway may result in defective development of the other pathway. In this study, the lack of RL-stimulated hypocotyl hair development in the phytochrome-deficient *au* mutant may result from deficiency in phytochrome. Since *au* has very few hairs on its hypocotyl, it is likely that a diminished amount of the Pr form of phytochrome may have caused this effect. It has been shown that in *phyB-1* mutant of *Arabidopsis*, absence of phyB leads to abnormal elongation of root hairs in the mutant. It is therefore possible that initiation and elongation of hypocotyl hair in tomato may be mediated by different phytochrome species.

Since *au* possess maximal deficiency of phytochrome in reduction of phyA, the factor responsible for placing of hair in tomato might be under the control of phytochrome A. At the same time, it is also possible that *au* locus is a common point of regulation for both accumulation of
functional phytochrome A and for the expression of the factor responsible for hair density. Since dark-grown seedlings of au have fewer hairs when compared to dark-grown WT, it is possible that lack of phytochrome activity in au may lead to decline in hair density too. However, it is also likely that such an effect may arise due to the pleiotropic nature of au mutation.

The presence of a very high density of hairs on hypocotyl (Fig. 4.5) and accumulation of high amounts of anthocyanin (Table 4.1) in hp mutant is analogous to the phenotype of the transgenic Arabidopsis mutant which overexpresses the R gene of maize. It is known that anthocyanin induction and hair initiation may be jointly governed by the action of the R and TTG genes. Furthermore, it is reported that the R gene of maize can rescue the ttg mutant of Arabidopsis which is defective in trichome initiation, by providing TTG function (Lloyd et al., 1992). This may well be the case in WT plants, where the HP gene product may counter the action of the R gene, which is believed to be the universal regulator of anthocyanin induction (Goff et al., 1992). The decline in the level of HP product in hp mutant leads to high levels of anthocyanin, and high hair density on hypocotyl. Therefore, the molecular characterization of HP locus in tomato may lead to the isolation of a negative regulator in the photomorphogenic pathway of the above responses.

5.2 Role of phytochrome in cotyledon expansion

Cotyledon expansion in dicots, marked by an increase in size and accumulation of fresh mass, is stimulated by light (Sangeetha and
Sharma 1988). The cotyledon expansion in seedlings with genetically lower levels of phytochromes was severely reduced when compared to WT in continuous RL, indicating a role of phytochrome in cotyledon expansion under these conditions (Neff and Volkenburgh 1994).

In RL-grown au seedlings the process of cotyledon expansion was severely inhibited; cotyledons accumulated only a third of the fresh mass of WT. At the same time, au cotyledons grew normally under WL. The inefficiency of au cotyledon expansion under RL clearly indicates that while residual phytochrome (Sharma et al., 1993) can induce several enzymes in au which may be via phyB or other phytochromes, severe deficiency of phyA restricted the cotyledon expansion in it. This is also supported by recent observation in fri mutant of tomato which is a null PHYA mutant where an efficient chlorophyll biosynthesis and cotyledon expansion was observed under WL, but not in the seedlings pretreated with FR before transfer to WL (Van Tuinen et al., 1995a) suggesting a role for phyA in cotyledon expansion. This is contrary to the findings in Arabidopsis where the light-mediated expansion of cotyledons is attributed to phyB (Neff and Volkenburgh 1994). Similarly, the EODFR response, considered to be a phyB mediated response, is absent in phyB mutant of Arabidopsis (hy3) (Whitelam and Harberd 1994), but present in phyB mutant of tomato (tri) (Van Tuinen et al., 1995b). These observations indicate that similar phytochrome forms in different species may perform different roles, depending upon the growth conditions and nature of the surroundings of the plant species.
Studies with BL mutants of *Arabidopsis* have shown that the process of cotyledon expansion is also mediated by BL (Blumm et al., 1994). The restoration of cotyledon expansion in *au* under WL may be as a result of the co-action of the BL-receptor and residual phytochromes. A similar kind of rescue of other photoresponses like induction of transcripts of certain plastidic proteins (Oelmuller and Kendrick 1989), induction of NiR activity (Fig. 4.34), chlorophyll accumulation (Fig. 4.7), and cotyledon expansion (Fig. 4.3) was observed in *au* mutant under WL.

5.3 **PAL induction in tomato is mediated by a stable pool of phytochrome**

Tomato WT seedlings grown under light characteristically accumulate anthocyanin in several regions of seedlings particularly in the hypocotyl hook, and at the junction near the root. However, *au* seedlings completely lack this light-mediated anthocyanin induction. In most systems it is observed that the photoinduction of anthocyanin is preceded by an enhancement in the PAL level. (Beggs et al., 1986, 1987; Brodenfeldt and Mohr 1988; Hahlbrock and Scheel 1989). In view of the causal interrelationship between anthocyanin accumulation and regulation of PAL, we examined the photoregulation of PAL in phytochrome-deficient *au* mutant, hypersensitive *hp* mutant, and in *au, hp* double mutant of tomato.

In tomato, RL initiates a strong photoinduction in PAL activity (Figs. 4.13 and 4.14) and PAL protein (Figs. 4.19 to 4.21) in both the hypocotyl and the cotyledons. This photoinduction of PAL in tomato can
be attributed to phytochrome, since the increase in PAL activity by a brief RL pulse is reversed by a subsequent pulse of FR (Lercari et al., 1982). Interestingly, this photoresponse is observed in all the tomato mutant seedlings, even though the au and au,hp mutants possess no spectrally active phytochrome in etiolated seedlings (Sharma et al., 1993). The close similarity in the time course of photoinduction of PAL activity in both au and au,hp mutant to WT indicates that the deficiency in Phytochrome A does not impair the photoregulation of PAL in these mutants. The sustainment of PAL activity at a higher level in the cotyledon and greater magnitude of photoinduction of PAL in the hypocotyl in hp mutants are in accordance with it being a signal transduction amplification mutant (Adamse et al., 1988b, Peters et al., 1989). The sustainment of a high level of PAL in cotyledons of hp mutants may result from either a reduced rate of PAL degradation or a sustained synthesis of PAL at a steady level. In the hp mutant there is a general increase in the PAL level since even in total darkness it is double than that in the WT seedlings.

In seedlings of the au genotype the R/FR reversible photore sponses are lacking or severely reduced at the stage of de-etiolation, presumably due to significant reduction in the level of phytochrome A in etiolated seedlings (Sharma et al., 1993). In the present study, it is evident that the FR reversible response on the PAL level in both the hypocotyl and in the cotyledons of au and au,hp mutant is nearly equivalent to that in WT seedlings. In previous studies with au seedlings, such a reversal of an inductive RL pulse by FR has not been observed, except for the photoregulation of CAB gene expression (Sharrock et
al., 1988; Oelmiiller and Kendrick 1991) which is however greatly reduced in magnitude compared with WT. Since au is deficient in phytochrome A it is possible that the above PAL response is mediated by the other phytochrome species constituting the stable pool, which has been shown to accumulate in light-grown au plants (Adamse et al., 1988a), and which regulates photoresponses in a fashion similar to light-grown WT plants (López-Juez et al., 1990). Out of different phytochrome species constituting the stable pool, at least spectrally active phytochrome B apoprotein levels were shown to be equal to WT (Sharma et al., 1993) and may be involved in PAL induction. But, due to lack of monoclonal antibodies to other forms of phytochromes such as C or D in the stable pool, it is difficult to say which phytochrome species of the stable pool is mediating the observed induction of PAL in au. Another possibility, of a low residual phytochrome labile pool present below detection limits mediating PAL induction, can be completely excluded because it was shown that though the level of PHYA polypeptide in etiolated au seedlings was about 20% that of WT, it did not show any spectral activity and failed to reconstitute into spectrally active phytochrome in presence of phycocyanobilin both in vitro and in vivo. The observed R/FR reversible phytochrome response in au, similar to the magnitude of WT response, argues against the notion that au seedlings are almost red-blind and therefore do not completely de-etiolute under RL.
5.4 Photo-induction of PAL and anthocyanin accumulation are not correlated

The photoinduction of PAL in tomato seedlings does not show good correlation with stimulation of anthocyanin biosynthesis in the same seedlings (Adamse et al., 1989). In the \textit{au} and \textit{au, hp} seedlings, little anthocyanin is produced, but the time course of increase in PAL level in respect to RL is similar to WT. Similarly, in the hypocotyl, a correlation between PAL induction and anthocyanin level is not evident. In tomato seedlings, the majority of anthocyanin biosynthesis takes place in the hypocotyl and its decrease is completed within 8 h, before the onset of the sustained increase in the anthocyanin level (Peters et al., 1991). Although RL irradiation leads to a greater enhancement in the level of PAL in the hypocotyl of \textit{hp} mutants, PAL activity is also significantly enhanced in WT, \textit{au}, and \textit{au, hp} mutant seedlings which fail to produce much anthocyanin (Table 4.1). Furthermore, in \textit{au} seedlings a single pulse of RL significantly increases PAL level, but the same \textit{au} seedlings do not show any detectable level of anthocyanin with a single RL pulse (Adamse et al., 1989). Moreover, in all the genotypes used except the \textit{hp} mutant, a blue light pretreatment is required to observe the phytochrome regulation of anthocyanin induction (Adamse 1988; Mancinelli 1985) while in the present study the genotypes used exhibited R/FR reversible photoregulation of PAL without a blue light pretreatment.

It is evident from the foregoing discussion that there is no strict correlation between the photoinduction of PAL measured here, and the biosynthesis of anthocyanin in tomato seedlings. The lack of correlation between photoinduction of anthocyanin biosynthesis and PAL has been
noticed in other systems on the basis of lack of coordination in the kinetics of photoinduction of PAL and anthocyanin accumulation (Brodenfeldt and Mohr 1988), while our studies rule out a direct correlation between a phytochrome-mediated induction of the major pool of PAL and anthocyanin biosynthesis. But, they do not rule out a key role played by PAL in anthocyanin biosynthetic pathway, since the inhibition of PAL activity in vivo by \( \alpha \)-aminoxy, \( \beta \)-phenyl propionic acid, strongly reduces the photoinduction of anthocyanin in tomato seedlings (Table 4.1).

PAL activity is a prerequisite for anthocyanin synthesis. In mustard seedlings where anthocyanin is localized in the lower epidermis of cotyledons, after dissecting cotyledons into lower and upper parts, Beggs et al. (1987) found a correlation between PAL and anthocyanin increase in the lower epidermis during the first few hours of anthocyanin accumulation. Since anthocyanin in tomato seedlings is also strictly localized in the lower epidermal layer of the cotyledons and the sub-epidermal layer of the hypocotyl, it is possible that PAL activity in these epidermal layers follows a different kinetic pattern of photoregulation than the total pool in the whole organs. The inhibition of anthocyanin accumulation by a-aminoxy, \( p \)-phenyl propionic acid (Table 4.1) indicates that a minor pool of PAL may play a role in anthocyanin accumulation. However, such tissue-specific correlation between photoregulation of PAL and end product accumulation has not been firmly established for PAL. Another enzyme, chalcone synthase, which plays an important role in regulating anthocyanin synthesis, clearly
shows a tissue specific distribution and induction by light (Ehmann et al., 1991; Schmelzer et al., 1988).

The inhibition of PAL photoinduction by RNA and protein synthesis inhibitors (Figs. 4.17 and 4.18) indicates that PAL is synthesized de novo in tomato seedlings (Brodenfeldt and Mohr 1986). This was further confirmed by western blotting using tobacco PAL antibodies, which showed the accumulation of PAL protein till 6 h of irradiation in au (Fig. 4.20) and WT (Fig. 4.19), whereas in hp (Fig. 4.21), under RL, higher PAL levels were observed till 12 h of irradiation and decreased by 24 h in cotyledons and hypocotyls of all the mutants and WT seedlings.

From the observations of the present study, it is evident that although phytochrome mediates PAL induction, the major pool of PAL present in tomato seedlings does not participate in controlling the anthocyanin level upon exposure of etiolated seedlings to continuous RL. The results presented in this study also indicate that while the photoregulation of anthocyanin synthesis is dependent on phyA and the photoregulation of PAL is proposed to be mediated by the stable pool in tomato seedlings. But, on contrary to this the PhyB deficient tri mutant of tomato also accumulates less amounts of anthocyanin in seedlings grown under RL suggesting a role for PhyB also.

This paradox can be resolved by studying the PAL induction and anthocyanin biosynthesis in a proven PHYA and PHYB null mutants like fri (Van Tuinen et al., 1995a) and tri (Van Tuinen et al., 1995b) mutants along with the doubly null mutants of PHYA and PHYB. These studies
will also help in identification of certain exclusive and some overlapping functions of both PHY A and PHY B.

### 5.5 A urea mutant retains photoinduction of cytosolic enzymes, but lacks photoinduction of plastidic enzymes

Earlier studies on RL-mediated photoinduction of several nuclear encoded plastidic genes revealed that *au* seedlings lack photoinduction of plastidic proteins and need blue light to elicit Pfr action on these proteins; but under white light, *au* plants survive and complete their life cycle normally (Oelmüller and Kendrick 1991). By contrast, our results indicate that even brief pulse of RL can induce PAL activity in *au* seedlings. Since PAL is a cytosolic enzyme, it would be quite logical and interesting to know whether other cytosolic enzymes are also similarly induced by RL in *au* and nature of the RL-mediated photoinduction of plastidic enzymes in *au*.

The results obtained with *au* in this study are in accordance with the fact that the level of physiologically functional phytochrome is severely reduced in etiolated seedlings of this mutant. Although the dark-grown seedlings of *au* possessed a basal level of amylase (Fig. 4.23), NR (Fig. 4.24), and NiR (Fig. 4.25) activity, a brief RL pulse did not stimulate any of these enzymes in *au*, but stimulation was observed in WT and *hp* which was reversible with FR pulse. The absence of RL pulse-mediated enzyme induction in *au* is consistent with previous studies, demonstrating that *au* shows little induction of photoresponses with RL pulses (Oelmüller et al., 1989; Oelmüller and Kendrick 1991; Becker et al., 1992). Since PAL in *au* can be induced by brief RL pulses,
and NR and NiR do not respond to the same pulse, it is evident that different enzyme inductions require different amounts or fluence to initiate response, PAL being more sensitive than NR and NiR. Although $au$ did not respond to brief RL pulses, it showed a stimulation of amylases and NR activity under continuous RL, in a fashion qualitatively similar to WT and $hp$ seedlings. This stimulation can also be attributed to phytochrome because in addition to R/FR reversible low-energy response, the continuous RL mediated high-irradiance response also represents a criterion for the involvement of phytochrome, and in case of $au$, it may be one or more of the stable pool phytochrome species.

The time course of NR (Figs. 4.26 and 4.27) and amylase (Figs. 4.28 and 4.29) induction in $au$ under continuous RL followed profiles similar to those in WT and $hp$, except that the magnitude of enzyme induction in $au$ was considerably lower than in WT and $hp$. Evidently, barring the absence of NiR photoinduction in $au$, the deficiency of phytochrome in $au$ or amplification of sensitivity to phytochrome in $hp$ did not influence the profiles of enzyme induction, such as the duration of lag or the time required to attain peak induction of enzymes. The higher magnitude of photoinduction of enzymes in $hp$ seedlings are in conformity with the observed pleiotropic effect of $hp$ mutation on amplification of phytochrome-regulated responses (Peters et al., 1992). By contrast, the observation that continuous RL stimulates amylase, NR, and PAL activities in $au$, is at variance with previous studies where continuous RL-mediated induction of several nuclear transcripts could not be detected in etiolated $au$ seedlings (Sharrock et al., 1988; Oelmtüller et al., 1989; Oelmtüller and Kendrick 1991).
The retention of continuous RL-mediated induction of NR and amylase along with PAL in seedlings, despite severe reduction in the spectrally active phytochrome A level, indicates that au has a residual active phytochrome pool that regulates the above responses. Physiological experiments have shown that mature plants of au retain the EODFR response (Adamse et al., 1988; López-Juez et al., 1990) and shade-avoidance reactions (Whitelam and Smith 1991; Kerckhoffs et al., 1992) which are assumed to be mediated by a photostable phytochrome. Since mutants deficient in PHYB apoprotein (López-Juez et al., 1992; Reed et al., 1992, 1993) lack the above responses, it is assumed that at least PHYB is functional in mature au plants. This was biochemically confirmed by showing that the elution profile of spectrally active phytochrome from green leave of au was similar to that of PHYB, indicating PHYB is spectrally active (Sharma et al., 1993). Considering the existence of spectrally active phytochrome in green au plants and the retention of phytochrome triggered responses in au seedlings, it is plausible that the residual phytochrome pool of au seedlings may consists of photostable phytochrome species. However, as discussed, under the PAL induction the relative proportion and functional contributions of phytochrome species constituting the stable phytochrome pool in au are not known because of the lack of monoclonal antibodies to other individual phytochrome species.

5.6 Chloroplast development in aurea mutant

Although it has been tacitly assumed that defective photo-regulation in au arises from phytochrome deficiency, the pleiotropic
nature of the *au* mutation cannot be ignored. Though it has been suggested that *au* is a PHYA deficient mutant (Sharma et al., 1993; Parks and Quail 1993; Whitelam et al., 1993), *au* plants grown under normal day light differ from WT, being pale green in color (López-Juez et al., 1990; Becker et al., 1992) In comparison, the phenotype of PHYA null mutants of both Arabidopsis (Nagatani et al., 1993) and tomato (Van Tuinen et al., 1995a) are indistinguishable from WT. Unlike WT, *au* possess a agranal Chloroplast with a reduced number of thylakoid membranes (Koornneef et al., 1985). Such a pleiotropic effect of *au* mutation on Chloroplast development indicates that in addition to phytochrome deficiency, the lack of a functional Chloroplast, or defective Chloroplast may also influence expression of nuclear encoded genes like NiR (Oelmüller et al., 1989). For instance, it has been shown that expression of nuclear genes that encode plastidic proteins like CAB and RBCS is closely associated with Chloroplast development (Susek et al., 1993). In the present study, although photoinduction of cytosolic enzymes such as amylase and NR are present in *au*, a similar induction of plastidic proteins like NiR and of mRNA levels for other plastidic proteins (Sharrock et al., 1985; Oelmüller and Kendrick 1991) is not seen. The absence of photoinduction of NiR in *au*, even under continuous RL, is an intriguing observation because continuous RL stimulated accumulation of NR and NiR transcripts. Becker et al. (1992) showed that although a brief RL pulse failed to elevate the NiR and NR transcript levels in *au* continuous RL elevated both NR and NiR transcripts. In contrast, RL mediated increase in NR transcript level (Becker et al., 1992), and stimulated de novo synthesis of NR in *au* (Figs. 4.34 to 4.37) as revealed by tungstate mediated inactivation of newly
synthesized NR molecules (Deng et al., 1989); a similar increase in NiR transcript level in \emph{au} is not accompanied by a stimulation in NiR enzyme level.

It is likely that the observed discrepancy between the RL-mediated increase in NiR transcript level and the absence of photoinduction of NiR activity may arise from a block in Chloroplast development in \emph{au}. Although phytochrome induction of NiR transcript (Becker et al., 1992) may not be tightly linked with Chloroplast differentiation, but because it is a plastidic enzyme, the expression of NiR activity is likely to be dependent on Chloroplast development. Neuhaus et al. (1993) has unequivocally shown that in hypocotyl cells of etiolated \emph{au} seedlings, plastid development is arrested at the level of proplastids which do not even differentiate into etioplasts. Moreover, these proplastids do not transform to chloroplasts even after a 48 h exposure to WL. In the present study also, it was demonstrated that cotyledons of \emph{au} seedlings possess a delayed and sluggish acquisition of photosynthetic oxygen evolution by monitoring the capacity of chloroplasts to acquire \textit{in vivo} light-mediated carbon dioxide dependent oxygen evolution. This also suggested that the \emph{au} mutant is impaired in photoinduction of Chloroplast differentiation. It is possible that the above delay in Chloroplast development in \emph{au} may in some way be responsible for the absence of photoinduction of NiR. The observation that NF induced photo-oxidation of chloroplasts, drastically reduces the NiR levels in wild type and \emph{hp} indicating that the chloroplast's integrity is essential for photostimulation of NiR activity (Oelmüller et al., 1989). The above discussion highlights the fact that even though \emph{au} retains
photinduction of enzymes that are possibly regulated by residual phytochrome, deficiency in NiR photoinduction may be either due to PHYA deficiency or due to Chloroplast development.

**5.7 Role of BL in au development**

Since *au* mutants survive despite being deficient in phytochrome and having impaired Chloroplast biogenesis, it is possible that co-action of another photoreceptor during de-etiolation may alleviate the adverse affects of phytochrome deficiency. For example, BL pretreatment of *au* restores phytochrome mediated induction of nuclear transcripts encoding plastidic proteins (Oelmüller and Kendrick 1991). Although NiR is a nuclear-encoded plastidic protein, a BL pretreatment of *au* did not induce NiR activity, whereas BL largely replaced RL-mediated NiR induction in *hp* and WT. Only when *au* seedlings were exposed to continuous WL could a reduced level of NiR photoinduction be observed. likewise photoinduction of RBCS transcripts in *au* was seen only under continuous WL in *au* (Sharrock et al., 1988). Probably a simultaneous operation of BL photoreceptor and residual phytochrome under continuous WL restores photoinduction of NiR by stimulating Chloroplast development in WL seedlings.

**5.8 General conclusions**

The physiological studies so far carried out in *au* mutant revealed several abnormalities right from the process of germination to the completion of its life cycle, but the actual reason for the observed deficiencies is still not precisely known. At the seedling stage *au* mutant
exhibit more pleiotropic phenotype compared to WT due to phytochrome
deficiency which is characterized by reduction in

(i) hypocotyl growth inhibition in WL, FR, R, B, and UV-A
(Koornneef et al., 1985; Adamse et al., 1988),

(ii) Chlorophyll and Chloroplast development (Koornneef et al.,
1985; Ken Dror and Horwitz 1990; Neuhaus et al., 1993)

(iii) Anthocyanin content (Adamse et al., 1989)

(iv) The photoregulation of the transcript levels of chlorophyll a/b
binding proteins of PSI and PSII, plastocyanin and subunit II of
photosystem I (Sharrock et al., 1988; Oelmüller and Kendrick 1991).

Adding to this list, the present work has identified

(i) the reduced photoinduction of cytosolic enzymes like PAL, NR,
amylase

(ii) total loss of photoinduction of plastidic enzyme NiR

(iii) reduced cotyledon expansion, chlorophyll accumulation and
photosynthetic oxygen evolution under RL, and

(iv) presence of short and low density of hairs on hypocotyl.

Analysis of the results obtained earlier and the present data suggest
that in au mutant, phytochrome deficiency is targeted more towards
phyA than other phytochromes. However, the recent isolation and
characterization of type-specific phyA and phyB mutants in tomato led to
some contrasting suggestions. For example, the phyA deficient fri mutant
was selectively insensitive to FR (Van Tuinen et al., 1995a) and phyB
deficient tri mutant is insensitive to RL alone (Van Tuinen et al., 1995b)
whereas, the lack of responsiveness of the au mutant to both R and FR is
pointing it towards being a chromophore mutant. At the same time,
earlier evidence such as presence of reduced levels of immunochemically
detectable phyA, WT levels of spectrophotometrically detectable
phytochrome, and immunochemically detectable phyB (Sharma et al.,
1993) in au mutant and their absence in well established chromophore
mutants strongly suggest that au is not a true chromophore mutant. Both
phyA and phyB1 deficient mutants of tomato exhibited normal EODFR
responses, indicating that either phyB2 or other phytochrome forms
mediate this response. The presence of

(i) normal EODFR response (López-Juez et al., 1990)

(ii) normal response to very high reduction in R:FR photo ratio
(Kerckhoffs et al., 1992) though less sensitive response to very low
changes in R:FR ratio than WT (Casal and Kendrick 1993), and

(iii) R/FR reversible induction of PAL activity
in au mutant indicate that au does have functional phytochrome species
which cannot be expected from a true chromophore mutant. Moreover,
the exogenous supply of chromophore could restore the WT phenotype
in all the chromophore mutants of Arabidopsis (Parks and Quail 1991)
and tobacco pew 1-1 mutant (Kraepiel et al., 1994). In contrast, in au
mutant, both in vivo and in vitro attempts to rescue au phenotype and au
polypeptide by exogenous supply of chromophore were unsuccessful
(Reedy and Sharma, personal communication), as in the case of pew2-l
mutant of tobacco.

The pew2-l mutant of tobacco to a great extent is similar to au
mutant of tomato: first, it does not show rescue by exogenous biliveridin,
second, it produces reduced levels of phyA polypeptide, and third, it has
a normal level of light-stable phytochrome when grown under WL. In
contrast, *pew2-1* mutant, which is a true chromophore mutant, possess normal levels of phytochrome apoprotein in dark and in light-grown plants but the level of spectrally active phytochrome is severely reduced. Taking these data together we suggest that since tobacco and tomato belong to same solanaceae family, both *pew2-1* locus and *au* locus may have something in common, and may represent a mutation distinct from normal chromophore mutants.

Though it may be debatable whether *au* is a true phyA deficient mutant or not, Neuhaus et al. (1993) presented evidence that microinjection of purified phytochrome A into hypocotyl cells of *au* mutant complements the *au* phenotype by inducing anthocyanin accumulation and Chloroplast development. It is suggested that though *au* is not a null phytochrome mutant, the roles of PhyA deduced from the physiological analysis of *au* can be assigned to phyA and the signaling intermediates identified by its biochemical analysis can be considered part of the phyA signal-transduction pathway. From our observation we speculate that reduction in the level of PHYA polypeptide in *au* may be because of a defect in a more general process such as translation, or a defect in some post-translational modification step specific for phyA leading to the lack of bilin C-S lyase activity and increased degradation of the protein.

Despite the deficiencies such as

(i) 30-40% decreased chlorophyll and RuBPCase content (Becker et al., 1992),
(ii) reduction in the number of thylakoids in Chloroplast (Koornneef et al., 1985; Neuhaus et al., 1993),

(iii) deficiency of protochlorophyllide in dark grown seedlings (Ken-Dror and Horwitz 1990), and

(iv) being defective in both abundance and light regulation of light-harvesting chlorophyll a/b binding polypeptides (Ken-Dror and Horwitz 1990),

the maximum photosynthetic rate of the \( au \) mutant is only slightly reduced. The \( au \) mutation does not result in damage or marked loss of efficiency in the photosynthetic electron-transport system under continuous WL (Becker et al., 1992). However, the net leaf photosynthesis under vegetation shade is significantly affected by the phytochrome deficiency but the \( au \) lesion has little effect on the capacity of tomato to exhibit photoadaptation to stimulated vegetation shade when measured at the thylakoid level (Smith et al., 1993). The comparison of the photosynthetic performance of four week old plants and flowering plants indicate that the impact of the \( au \) mutation on growth and development becomes reduced with increasing age of the mutant plants (Becker et al., 1992).

The survival of \( au \) mutant in normal daylight and restoration of RL-impaired responses to WT levels under WL such as fresh mass accumulation, chlorophyll accumulation, photosynthetic oxygen evolution, and NiR photoinduction, suggest that a simultaneous operation of a BL receptor and residual phytochrome may be restoring the above responses under WL and may be helping the mutant to complete its life cycle. Such a type of interdependent co-action (Mohr
between phytochrome and specific BL/UVA receptor was shown in the control of stem extension growth responses to BL in au mutant (Casal 1994). It was also suggested that au mutant is deficient in a pool of phytochrome apparently not essential for the interdependent co-action between phytochrome and BL/UVA receptor observed for stem extension growth (Casal 1994). Furthermore, the observation of a BL-promoted in vitro bud regeneration both in the presence of high and low IAA concentration in au mutant (Mercenaro et al., 1994), indicates the presence of functional BL receptor in it.

The hp mutant of tomato is characterized by the presence of phytochrome content of etiolated seedlings (predominantly phytochrome A) and the physiological characteristics of phytochrome system similar to that in WT (Adamse et al., 1989; Peters et al., 1989). At the same time it exhibits exaggerated phytochrome responses such as maximum anthocyanin synthesis, and hypocotyl growth inhibition in RL alone, and does not require co-action of the BL photoreceptor and phytochrome for normal development. The results in the present thesis indicate that hp mutant is quite opposite to au mutant with respect to photoresponses which were deficient in au such as high dark levels of all enzyme activities assayed, higher magnitude induction of all the enzymes studied high amount of chlorophyll, high amount of fresh mass accumulation longer, and higher density of hairs on hypocotyl, suggesting that the phytochrome species whose responsiveness is amplified in the hp mutant and the phytochrome species which is deficient in au may both be same. Furthermore, the observation of normal phytochrome responses such as hypocotyl elongation growth in response to EODFR treatment and low
R:FR ratio in both *au* and *hp* mutants will further strengthen the above suggestion. Therefore, the molecular analysis of *hp* mutation may result in identification of a signaling component specific to a phytochrome species that is deficient in *au*, which is predominantly phytochrome A.