Chlorophyll is the most abundant tetrapyrrole in the plants. It plays an essential role in the absorption of light during photosynthesis. Chlorophyll turnover is very important for proper functioning of photosynthetic apparatus under normal as well as in different environmental conditions. To maintain chlorophyll turnover its biosynthesis and degradation should be highly regulated. During senescence and fruit ripening, chlorophyll a is hydrolyzed in to pheophorbide or chlorophyllide (Chlide) and free phytol. However, phytol produced during chlorophyll degradation is usually recycled for chlorophyll resynthesis using chlorophyllide as a substrate by the enzyme Chl synthase (Chl G). This recycling conserves several energy resources including NADPH. The phytol before esterification with chlorophyllide is phosphorylated by phytol kinase (VTE5) to phytol phosphate. The latter is further phosphorylated to phytol diphasphate by an yet unidentified kinase. The phytyldiphosphate, either recycled from chlorophyll catabolism or synthesized de novo, and chlorophyllide (Chlide), newly synthesized by protochlorophyllide oxidoreductase (POR) via photoreduction of protochlorophyllide (Pchlide), are esterified by chlorophyll synthase to form new chlorophyll molecules. Phytyldiphosphate could also be metabolized to tocopherol by homogentisate phytlytransferase. Therefore, phytol kinase acts as a key enzyme at the interface of chlorophyll biosynthesis and tocopherol biosynthesis and serves as a connecting enzyme between chlorophyll anabolic and catabolic pathways.

The tocopherols are efficient quenchers of $^{1}{\text{O}}_{2}$. As tocopherols are highly hydrophobic they are located in the membrane bilayer and protect the biomembranes from $^{1}{\text{O}}_{2}$-induced injury.

Genetic manipulation of chlorophyll and tocopherol biosynthesis in Arabidopsis and Brassica via overexpression of Phytol kinase (VTE5) augments the amount of chlorophyll and tocopherol (vitamin E). Increased chlorophyll synthesis coupled with tocopherol mediated quenching of $^{1}{\text{O}}_{2}$ and other reactive oxygen species leads to increased photosynthesis, plant productivity and grain yield in normal growth conditions as well as in a stressful environment. Similarly to minimize $^{1}{\text{O}}_{2}$ generation, protochlorophyllide oxidoreductase C (PORC) was overexpressed in Brassica. This could protect plants from $^{1}{\text{O}}_{2}$-induced oxidative stress caused by photodynamic herbicide and salinity.

**Genetic manipulation of AtVTE5 in Arabidopsis thaliana**

Vitamin E pathway gene 5 (VTE5) encodes phytol kinase which is responsible for reutilization and activation of phytol, generated during chlorophyll catabolism. Bioinformatic
analysis of deduced amino acid sequence of $AtVTE5$ reveals that it is a transmembrane protein having 6 transmembrane helices. Phytol kinase is a chloroplast targeting enzyme having phosphatidate cytidylyltransferase activity. Its homologous sequences are also present in archaea, eubacteria, cyanobacteria and plants. Study of tissue specific expression of $AtVTE5$ reveals its maximal expression in old leaves which are in senescence stage having increased chlorophyll degradation. $VTE5$ expression was minimal in 1$^{st}$ week and increased after 7$^{th}$ week. These expression studies clearly demonstrate that $AtVTE5$ expression is developmentally regulated and its expression increases during senescence when chlorophyll degradation is in progress.

Transgenic approach was followed to show the functional significance of $AtVTE5$. The $AtVTE5$ transgenic Arabidopsis plants having sense and antisense expression under constitutive 35S promoter were raised. $AtVTE5$ gene and protein expression substantially increased in sense plants and decreased in anti$VTE5$ plants. $AtVTE5x$ plants were bigger in size, flowered earlier and had longer root than that of WT plants whereas anti$VTE5$ plants had similar phenotypic growth as compared to that of WT. Fresh weight, dry weight and floral diameter were also higher in $AtVTE5x$ plants compared to WT and anti$VTE5$ plants. The chlorophyll contents of $AtVTE5x$ plants were higher (18%) than that of WT. However, in anti$VTE5$ plants chlorophyll contents were lower (17%) than that of WT. Increased chlorophyll synthesis in $AtVTE5x$ plants may be due to increased esterification of chlorophyllide, a photodynamic singlet oxygen generating compound, and phytol to chlorophyll. Decreased production of phytlyldiphosphate, an intermediate for chlorophyll biosynthesis in antisense plants led to downregulation of chlorophyll biosynthesis. Increased production of phytlylphosphate in transgenic plants resulted in huge i.e., 300% increase in total tocopherol contents in sense plants. All the measured components of different isoforms of tocopherol and especially that of most active a-tocopherol increased (2.7 fold) in the $AtVTE5x$ plants. In opposite vein, the downregulation of $AtVTE5$ expression led to reduced (34%) tocopherol contents in antisense plants. $AtVTE5x$ plants had higher (10-50%) percentage of seed germination and anti$VTE5$ plants had lower (6-24%) seed germination than that of WT plants in the presence of 100-200 mM NaCl. Similarly the increased tocopherol contents in sense plants alleviated the delay in germination of Arabidopsis in 200 mM salt-stress. It is due to increased or decreased tocopherol content in sense and antisense plants respectively. Seed germination assay and seed viability assay under stress condition clearly demonstrate the tolerance of $AtVTE5$ overexpression line and sensitivity of antisense line to salt stress as evident from higher chlorophyll content and better growth in sense plant.
and lower chlorophyll content and reduced growth in antisense plants compared to WT plants. Due to increased chlorophyll and tocopherol content photosynthetic efficiency of \( \text{AtVTE5x} \) plants was higher than that of WT plants. This was probably due to increased tocopherol content in sense plants resulting in reduced accumulation of reactive oxygen species (ROS), generated during salt stress. This could protect the D1 protein from accelerated degradation and maintain PSII active under stress condition. Conversely, due to decrease chlorophyll and tocopherol content in \( \text{antiVTE5} \) there photosynthetic efficiency declined. In antisense plant due to lower tocopherol production, reactive oxygen species (ROS) accumulation would have increased resulting higher D1 degradation and reduced electron transport especially in stressful environment.

**Overexpression of \( \text{AtVTE5} \) in \( \text{Brassica juncea} \)**

Our study in model plant \( \text{Arabidopsis thaliana} \) revealed that overexpression of \( \text{AtVTE5} \) could protect plants from deleterious effect of salt stress. To ascertain if the same could protect a crop plant, \( \text{AtVTE5} \) was overexpressed in \( \text{Brassica juncea} \) plants under the control of constitutive \( \text{Cauliflower mosaic virus} \) (\( \text{CaMV} \)) 35S promoter with omega enhancer. \( \text{VTE5x-2} \) and \( \text{VTE5x-3} \) lines had single integration and \( \text{VTE5x-1} \) line had twice integration of transgene in to host \( \text{Brassica} \) genome. Transgenic lines having single integration of transgene were used for further experiments. VTE5 protein was also significantly higher in transgenic plants than that of WT. As in \( \text{Arabidopsis} \), the overexpression of \( \text{AtVTE5} \) in \( \text{Brassica} \) resulted in increased (22%) chlorophyll contents. The total tocopherol and especially that of most active \( \alpha \)-tocopherol increased (81%) in \( \text{VTE5x Brassica} \) plants. Increase in tocopherol content protected the leaf discs of transgenic from salt-stress.

Vermiculite-grown one month old \( \text{VTE5x} \) plant treated with salt for 8 weeks showed tolerance. In WT plants the flowering was substantially delayed. The transgenic plants had better growth than that of WT plants. Chlorophyll and carotenoid contents, measured after 4 weeks of salt treatment, were higher in \( \text{VTE5x} \) plants than those of WT. The chloroplast ultrastructure of transgenic plants revealed tolerance to salt stress as evident from reduced structural distortion of thylakoids in transgenic plants. This clearly demonstrates that tocopherol, a lipophilic antioxidant associated to biomembranes, protect cell membrane from salt-stress-induced oxidative stress. This is evident from reduced production of \( ^1\text{O}_2 \) in the transgenic plants due to its efficient quenching by increased amounts of the most bioactive \( \alpha \)-tocopherol in the membranes. Fo, Fm and Fv/Fm of WT plants were decreased in salt stress. However, the transgenic plants always had higher Fv/Fm ratio in stress condition than that of
WT. There was no change in Fo and Fm of transgenic plants under same growth conditions. This was probably due to increased tocopherol content in transgenic plants resulting in reduced accumulation of reactive oxygen species (ROS), generated during salt stress. Increased tocopherol content is known to protect PSII reaction center D1 protein from oxidative stress. Presence of well-developed grana and stroma thylakoids in transgenic plants and higher chlorophyll content resulted in higher photosynthetic efficiency of the transgenic lines i.e., electron transport rate, quantum yield of PSII, photochemical quenching of chlorophyll fluorescence than that of WT plants after NaCl treatment. As generation of $^1$O$_2$ was lower in transgenic plants in stress condition, their lipid peroxidation was lower resulting in reduced malondialdehyde (MDA) production. As damage to cell membrane was smaller in transgenic plants, they had reduced electrolyte leakage.

$VTE5x$ plants of Brassica exhibited early flowering and fruit setting. Salt stress (150 mM, EC $\sim$ 17 dSm$^{-1}$) was applied to soil-grown potted WT and transgenic plants grown in greenhouse revealed that $VTE5x$ plants were tolerant to salt stress. After 4 weeks of stress the flowering was delayed by two weeks in WT plants. Under identical conditions the $VTE5x$ plants flowered. After 10 weeks of application of salt, both WT and transgenic had fruits although the fruiting was delayed in WT plants and they had reduced number of seeds per plant. This is due to presence of higher amounts of tocopherols in transgenic plants.

Overexpression of $AtVTE5$ in Brassica modulated photosynthesis and plant productivity. Net photosynthetic rate of transgenic plants was higher in control as well as in stress condition. Their quantum yield of CO$_2$ assimilation increased from 0.069 in WT to 0.076 in $VTE5x$ plants. The light-saturated net CO$_2$ assimilation rate was higher (11%) in $VTE5x$ plants in normal growth conditions. Increased rate of respiration in the transgenic plants enhance the light compensatation point and contributed to augmented crop growth. Due to salt treatment the light saturated photosynthesis rate declined (10%) in WT whereas in $VTE5x$ plants it was not significantly affected. The quantum yield of photosynthesis in WT and $VTE5x$ plants was not affected due to salt stress.

Higher rate of photosynthesis in normal growth conditions in $VTE5x$ plants resulted in increased plant productivity and grain yield (21%). In salt stress condition $VTE5x$ plants had higher grain yield (102%) and more number of seeds per plant than that of WT. Due to longer grain filling duration the unit seed weight was higher in transgenic plants in control as well as in stress conditions.

These results demonstrate that the quenching of ROS, specifically that of $^1$O$_2$ by membrane bound lipophillic compound $\alpha$-tocopherol could protect plant from oxidative
stress and contribute increased plant productivity and grain yield. This was more evident in VTE5x plants grown in salt environment where plant productivity, grain filling and grain yield were much higher than that of WT. However these transgenic plants need to be tested in actual field condition for the response to salt stress-induced oxidative stress.

**Overexpression of *AtPORC* in *Brassica* confers tolerance to ALA-induced oxidative stress**

In plants there is a strong regulation of chlorophyll biosynthesis leads to optimal accumulation of chlorophyll and heme biosynthetic intermediates. Because these interrediated are photodynamic in nature and endogenously generate reactive oxygen species after absorption of light. Triple excited forms of tetrpyrroles are produced in light that interact with molecular oxygen (O₂) and generate \(^{1}\text{O}_2\) via type II photosensitization reaction. To study the real effectiveness of protochlorophyllide oxidoreductase C (PORC) overexpression to minimize the \(^{1}\text{O}_2\) generation in light, protochlorophyllide (Pchlide) was over-produce by spraying the plants with 5-amino levulinic acid (ALA) that bypasses the tetrpyrrole-mediated regulatory feedback inhibition.

In present study, PORC was overexpressed in *Brassica juncea* under the control of constitutive Cauliflower mosaic virus (CaMV) 35S promoter with omega enhancer. Southern blot analysis confirmed the single integration of *AtPORC* gene in *Brassica* genome. Increased accumulation of *AtPORC* transcripts and PORC protein in *Brassica* PORCx plants, resulted in higher (19-23%) production of chlorophyll.

WT and PORCx-1 *Brassica* plants were sprayed with 2 mM, 3 mM and 5 mM concentration of 5-amino levulinic acid (ALA) and plants were kept in dark for 14 h to accumulate the Pchlide which act as a photosensitizer. Upon illumination (50 μmoles photons m\(^{-2}\) s\(^{-1}\)) for 48 h both WT and PORCx-1 plants treated with 2 mM of ALA survived, although the WT plants had more necrotic leaves than that of PORCx-1 plant. WT plants treated with 3 mM or 5 mM of ALA were highly damaged, whereas the PORCx-1 plants survived. After 7 days of light exposure both WT and PORCx-1 plants treated with 2 mM ALA survived although, WT plants had reduced growth than that of PORCx-1 plants. However, 3 mM or 5 mM of ALA, killed WT plants while PORCx-1 plants survived. The tolerance of PORCx plants to ALA treatment was due to the abundant amount of PORC protein that efficiently photoconverted excess protochlorophyllide (Pchlide) to chlorophyllide (Chlide) after 20 min of light exposure leading to reduced accumulation of photosensitizer Pchlide in light than that of WT plants. Reduced accumulation of Pchlide in the PORCx plants led to decreased
production of $^{1}$O$_{2}$ and minimal cell damage and no plant death. The Fv/Fm, ETR, $\Phi$PSII and qP of Chl a fluorescence declined in ALA-treated WT plants exposed to light for 1 h. Continued light exposure up to 2-6 h resulted in substantial decrease of the above parameters. Higher production of $^{1}$O$_{2}$ in ALA-treated WT plants damages photosynthetic apparatus and therefore could be a reason for substantial reduction in photosynthetic efficiency of ALA treated WT plants after light exposure. ALA induced photodynamic reactions caused membrane lipid peroxidation that resulted in accumulation of increased amounts of malondialdehyde (MDA) in leaf tissues of WT plants. In PORCx plants due to reduced lipid peroxidation smaller amounts of MDA was produced. Although the death and complete photobleaching of the WT plants occurred after 24 h of light exposure, the cell membrane was substantially damaged even after 2 h of light exposure as revealed by electrolyte leakage. In PORCx plants the damage to cell membrane was minimal.

It is concluded that overexpression of PORC results in efficient photo-transformation of Pchlide to Chlide that decreases Pchlide contents and consequently releases the Pchlide-induced feed-back inhibition of ALA biosynthesis. Furthermore, due to increased photo-transformation of Pchlide and enhanced rate of chlorophyll synthesis, Pchlide accumulation decreased and consequently, generation of $^{1}$O$_{2}$ was reduced. Therefore, the $^{1}$O$_{2}$-mediated photo-oxidative damage in ALA-treated PORCx plants was minimal. It was earlier shown that ALA application to plants could cause photo-oxidative damage and ultimately kill plants. The present study demonstrates that photo-oxidative damage and ultimate plant death caused by ALA could be substantially minimized by overexpression of PORC that limits the generation of $^{1}$O$_{2}$. This approach could be biotechnologically exploited further to use ALA as a commercial selective photodynamic herbicide.

**Overexpression of AtPORC in Brassica confers tolerance to salt stress**

The leaf discs from the Brassica PORCx plants showed significant tolerance against high concentrations of NaCl as compared to that of WT. Vermiculite-grown one month old Brassica PORCx plant irrigated with half-strength Hoagland solution+NaCl for 8 weeks showed significant tolerance. The transgenic plants had better growth than that of WT plants. The continued irrigation of WT plants with half-strength Hoagland solution along with 100 mM or 150 mM NaCl perished; 200 mM NaCl caused plant death faster. Under identical conditions, Brassica PORCx plants survived, flowered and had pods although their growth, development and fruit setting was substantially downregulated as compared to untreated controls. Chl and carotenoid contents, measured after 4 weeks of salt treatment, were higher
in Brassica PORCx plants than those of WT. Reduced structural distortion of thylakoids i.e., lack of swollen thylakoids and near-intact granal organization in Brassica PORCx plants revealed their tolerance to salt stress. Overaccumulation of POR results in efficient phototransformation of Pchlide to Chlide leading to reduced accumulation of photosensitizer Pchlide in Brassica PORCx plants. WT plants accumulated higher amounts of Pchlide than that of untreated controls. In response to salt stress, the degradation of Chl a is higher in WT plants as compared to that of Brassica PORCx plants. Therefore, the accumulation of photosensitizers Pchlide and pheophorbide a were smaller in the transgenic they had reduced 

\[ ^{1}O_2 \] production and consequent decrease in MDA production, electrolyte leakage and cell death. Increased generation of 

\[ ^{1}O_2 \] resulted in injury to the thylakoid membrane leading to decreased Fv/Fm ratio. Fo, Fm and Fv/Fm of WT plants decreased in salt stress. However, the transgenic plants always had higher Fv/Fm ratio in stress condition than that of WT. There was comparatively less reduction in Fo and Fm of transgenic plants under same growth conditions. This was due to efficient phototransformation of Pchlide to Chlide by abundant POR enzyme in transgenic plants resulting in reduced accumulation of reactive oxygen species (ROS) more specifically \(^{1}O_2\). Well developed and less distorted thylakoid in Brassica PORCx plants resulted in higher photosynthetic efficiency i.e., Electron transport rate (ETR), yield of photosystem II (\(\Phi PSII\)), photochemical quenching in transgenic plants than that of WT in stress condition as well as in normal growth conditions.

To understand the impact of salt stress on plant productivity and seed yield, WT and PORCx plants were grown in soil in greenhouse conditions and treated with salt (EC~17 dSm\(^{-1}\)). Brassica PORCx plants had more foliar growth and fruiting. Soil-grown potted Brassica PORCx plants showed tolerance to salt stress (150 mM, EC ~17 dSm-1). After 6 weeks of stress the flowering was delayed by one week in WT plants. Under identical conditions the Brassica PORCx plants flowered. After 10 weeks of application of salt, both WT and transgenic had fruits although the fruiting was delayed in WT plants. AtPORC overexpression in Brassica resulted increase in photosynthesis and plant productivity. Net photosynthetic rate of transgenic plants was higher (14%) in control as well as in stress condition. Higher productivity and per plant grain yield in transgenic plants was due to increased rate of photosynthesis in normal growth conditions. In salt stress condition Brassica PORCx plants due to their increased (22%) photosynthetic rate, had higher (115%) grain yield/plant and more number of seeds per plant than that of WT. It was due to their longer grain filling duration that resulted in higher unit seed weight.