計畫編號：BM05-00

計畫名稱:利用模式植物進行逆境生物學之功能性基因體與蛋白質體研究

計畫主持人：林讚標

計畫摘要(中)：

非生物逆境造成世界上農業方面廣泛的損失。這些個別的生物逆境，例如:乾旱，鹽或者高熱，都受到熱烈的研究。雖然已經累積了大量的知識，但是仍然有許許多多對逆境反應的基因，仍然沒有被研究過。在本團隊計劃中，我們將對乾旱與熱逆境相關的基因或蛋白質進行基礎的研究。在子計劃當中，有五個將探討乾旱逆境(子計劃:1, 2, 3, 4,和 5)，兩個與熱逆境有關(子計劃 6 和 7)，一個與病毒逆境有關(子計劃 5)。我們將使用功能性基因體，蛋白質體，結構生物資訊與植物生理的方法來研究這些特殊基因的功能。七個子計劃所用的研究方法實際上是互補的。乙烯反應因子 (ERF)家系已經被研究過知道它們參與了植物對生物與非生物逆境的反應。因此，這個家系是非常珍貴的資源，因為它有潛能可用於改善植物對逆境的耐受力。我們有四個子計劃，希望研究 ERF 家系的基因，即 AtERF4 基因的功能(子計劃 2)與 AtERF4 蛋白質的結構(子計劃 4)，At2g20880 在耐旱方面的功能(子計劃 1)和 At5g61600 在乾旱與病毒防禦的功能(子計劃 5)，這些都會加強子計劃之間的凝聚力與合作關係。我們從未形成如此一個堅強的團隊，針對一個共同的興趣，這將強化我們在植物逆境基礎研究方面的成果，並且對作物改良有所貢獻。

計畫摘要(英)：

Abiotic stress conditions cause extensive losses to agricultural production worldwide. Individually, stress conditions such as drought, salinity or heat have been the subject of intense research. Though a great deal of knowledge has been advanced, a large array of genes responsive to stresses is still unknown and unstudied. In this team project we would like to study the basic aspect of dehydration and heat stresses related genes and proteins. Five projects deal with the dehydration stress (sub-projects 1, 2, 3, 4 and 5), two with heat stress (sub-projects 6 and 7), and one with pathogen stress (sub-project 5). We will use functional genomics, proteomics, structure bioinformatics and physiological

approach to study the specific gene function. The approaches used by the 7 sub-projects are complementary. The ethylene response factors (ERFs) family members that have been functionally characterized are involved in plant responses to biotic or abiotic stresses. Hence, this family seems to be a valuable resource to evaluate in terms of its potential for engineering plant stress tolerance. We have four sub-project want to study ERF family genes, i.e., The function of AtERF4 gene (sub-projects 2) and protein structure of AtERF4 (sub-project 4), the function of At2g20880 in dehydration (sub-projects 1) and the function of At5g61600 in dehydration and pathogen defense (sub-projects 5). This will enhance coherence and cooperation among sub-projects for the common interest. We have never formed such a team strong enough like this one working on a defined biological problem which will strengthen our basic research while has a potential to contribute to agriculture improvement.

計畫編號：BM05-01

計畫名稱：阿拉伯芥轉錄子 At2g20880 參與乾旱反應之功能性分析

計畫主持人：林讚標

計畫摘要(中)：

植物在其生活史中常常遭遇許多生物性與非生物性之逆境。乾旱逆境會影響到不同種類不同功能之基因表現。調控基因表現大部分是位於轉錄階層且由轉錄因子所調控。根據 Kamiya et al. (2002)之微陣資料，我們挑選了一個由乾旱所誘導的基因，At2g20880 來進行研究。At2g20880 含有一個高度保守的 AP2 domain，屬於 DREB 亞科。在預備實驗中，我們發現本基因可因乾旱而誘導但不受高溫與 ABA 之誘導。此外我們也發現過度表達的植物對乾旱的耐受性要比也生的好很多，本研究中我們會是使用各種反式遺傳的研究方法來研究 At2g20880 的功能。（第一年）我們將研究組織專一性表現，細胞內的位置，突變株與過度表達植株的生理現象。（第二年）我們將利用 pull-down 與共同免疫沈澱的研究方式發掘任何可與 At2g20880 發生反應的蛋白質。（第三年）我們將利用微陣的技術來顯示 At2g20880 過度表達植株過度表現與受抑制的基因。並使用 RT-PCR 或北方墨點轉漬法去驗證這些表現發生變異的基因。最後我希望對此基因在遭受

逆境之下之分子功能獲得徹底的了解。

計畫摘要(英)：

Plants often meet many biotic and abiotic stresses through their life span. Assortments of genes with diverse function are influenced by dehydration stresses. Most of their gene products may function in stress responses and tolerance at the cellular level. The predominant mechanism for controlling plant gene expression is regulated at the transcriptional level and is mediated by transcription factors. According to the microarray data of Kamiya et al. (2002), we have selected a dehydration-induced gene, At2g20880 to study. At2g20880 contains one highly conserved AP2 domain and belong to the DREB subfamily. In a preliminary experiment we found that this gene was highly inducible by dehydration but not by high temperature and abscisic acid. In addition, we found overexpressed plant survived better than wild-type under water deficient condition. In this study we will use various reverse-genetic approaches to study the function of At2g20880. (the first year) We will study tissue specific expression, cell localization, the physiology of knock-out and over-expressed plant. (the second year) we will use pull-down and co-immunoprecipitation to study any protein would interact with At2g20880 in vitro and in vivo, respectively. (the third year) We would like to use microarray to reveal the up-regulated and down-regulated gene using At2g2080 over-expressing plants. Using RT-PCR or northern blot validate the expressed genes. Eventually I hope to explicitly understand the molecular function of this gene under stress.

計畫編號：BM05-02

計畫名稱：ERF 抑制因子在阿拉伯芥逆境反應之功能探討

計畫主持人：吳克強

計畫摘要(中)：

在植物的轉錄因子中，ERFs (ethylene-responsive element binding factors)屬于一個大的基因家族。 ERFs 在植物中專一性的存在,顯示他們參與植物特有的生長發育過程和植物與環境的相互作用。到目前為止,大部分的 ERF 蛋白被認為扮演著轉錄活化因子之角色,可促進一些含有 GCC box 或 CRT/DRE element 的抗逆境基因及抗病基因之表

現,不過也有一小群 ERF 蛋白扮演著轉錄抑制因子之功能。對於 ERF 抑制因子的生物功能仍然不清楚,因此在這個研究裡我們將說明 ERF 抑制因子的功能及其作用方式。我們將著重于研究其中一個 ERF 抑制因子(AtERF4), 因為 AtERF4 可以被環境中的鹽分逆境及乾旱逆境所誘導。我們將利用 T-DNA 插入突變體來研究 AtERF4 在阿拉伯芥對環境逆境反應之功效，鑑定出 AtERF4 的目標基因及與其有相互作用的蛋白質。既然 ERF 抑制因子專一性的存在於植物中,並且與其他轉錄因子也沒有序列上的相似性,深入研究 ERF 抑制因子將會發現他們在植物中獨一無二的作用方, 而且會讓我們更加了解環境因子對於植物基因表現之相關調控機制。環境逆境如乾旱, 鹽害和植物病害每年都會造成主要農作物的損失，因此更清楚了解 ERFs 如何調控基因表現將可以廣泛的運用在農作物之改良，尤其是作物對於環境逆境的耐受性方面。

計畫摘要(英)：

ERFs (ethylene-responsive element binding factors) belong to a large family of plant transcription factors that are found exclusively in plants. The plant-specific existence of ERFs suggests that they may be involved in the unique processes required for the integration of plants with environmental cues. Most of the ERF proteins identified so far were shown to act as transcriptional activators to up-regulate GCC box and/or CRT/DRE -containing genes involved in stress tolerance and pathogen resistance. However, a small subfamily of ERF proteins can act as transcriptional repressors. The biological functions of the ERF repressors are still unknown. The proposed research will elucidate the function and mode of action of ERF repressors. We will initially focus our study on AtERF4, an ERF repressor that can be induced by environmental stress such as salinity and drought. The role of AtERF4 in plant response to environmental stress will be studied by analyzing T-DNA insertion mutants in Arabidopsis. The target genes and interacting proteins of AtERF4 will be identified. Since ERF repressors are plant specific and share no sequence homology to other classes of transcription factors, understanding their function will likely to reveal their unique mode of action in plants. This study will lead to a better understanding of environmental regulation of gene expression in plants. Environmental

stresses such as such as extremes of temperature, drought, salinity and pathogens, cause significant crop losses on an annual basis. A better understanding of how ERFs modulate gene expression may have wide application of crop improvement, particularly in the field of environmental tolerance.

計畫編號：BM05-05
計畫名稱:ERF 基因在植物青枯病與缺水逆境防禦機制之功能性研究
計畫主持人：鄭秋萍
計畫摘要(中)：

植物在生長過程常遭受各種生物性及非生物性逆境，農作物產量也因逆境而造成巨大損失，全球為解決此問題而採取的作法對生態系統也造成了不可避免的傷害。事實上，植物為了存活於自然界中各種嚴苛的環境，已經發展出不同的防禦機制。因此，研究植物的防禦機制並將之應用於農業上，將發展永續農業，並得以保護生態系統。番茄青枯病是全球最嚴重的病害之一，但我們對於植物如何抵抗這類土壤傳播病的防禦機制了解仍極少。Ethylene-responsive element binding factors (ERFs)是植物特有的轉錄因子，在植物的逆境訊息傳遞反應中扮演中樞角色，因此，了解 ERFs 如何調控植物在逆境下的反應當然是重要且必需的。藉由微陣列分析技術，我們已在阿拉伯芥中找到一個會在青枯病菌感染時快速被誘導表現的未知功能的基因 At5g61600；另外，利用 virus-induced gene silencing (VIGS)的方法，我們也證實番茄中的兩個 ERF 基因（LeERF3 和 TRSF）參與抗青枯病的防禦反應中。為了進一步研究相關的植物防禦機制，在此計畫中，我們將會針對 At5g61600 和特定的番茄 ERF 基因進行功能性分析，預計進行一系列的研究，包括：在不同時間點及不同部位的表現情況、在細胞內的表現位置、利用 transient/transgenic overexpression 與 knock-out/down 等方法來證實不同的 ERFs 在植物青枯病防禦機制中的角色、以 transcriptomic 策略分析受其調控之目標基因。此外，由於青枯病與缺水逆境有許多共通點，我們也將透過與其他子計畫的合作，同時研究這些 ERFs 在植物缺水逆境中所扮演的角色。另外，由於我們已經建立了完善的青枯病的分析系統，本子計畫也可協助其他子計畫，分析其研究之目標基因是否在植物青枯病反應中扮演重要角色。透過整體研究計畫的整合，本子計畫預期將在植物對抗疾病與缺水逆境防禦機制上提供重要資訊，並期望可集結有利於農作物生物

科技發展之資源。

計畫摘要(英)：

Plant constantly encounters environmental stresses, including abiotic and biotic factors. The huge amount of agricultural efforts devoted to reduce the tremendous losses in crop productivity due to stresses has led to irreversible harm to our ecosystems. In nature, most plants possess various defensive machineries to ensure their survival in harsh environments. Therefore, elucidation of plant nature defense mechanisms and explore their potential applications on improvement of crop stress tolerance would serve as a useful strategy for agriculture sustainability and thus benefit protection of our ecosystems. Tomato bacterial wilt (BW) is one of the most complex and serious crop diseases worldwide. However, information on plant defense response to systemic infection of soil-borne pathogens is very limited. ERFs (ethylene-responsive element binding factors) are plant-specific transcription factors, playing a pivotal role in plant signaling transduction pathways switching extracellular signals into cellular responses, including biotic and abiotic stress responses. A better understanding about how these proteins regulate plant stress responses is thus important and would be highly desired. By carrying out transcriptomic analysis using a micoarray approach, we have identified an early BW-responsive ERF gene of unknown function, At5g61600. In addition, using a virus-induced gene silencing (VIGS) approach, we have also evidenced that two tomato ERF genes, LeERF3 and TRSF, are involved in natural defense response against BW. To elucidate plant defense mechanism further, this sub-project will study functions of At5g61600 and tomato ERF genes (B1a and B3 clusters) in plant disease defense, and gain insights into the involved mechanisms. Spatial/temporal gene expression and sub-cellular localization of the ERF gene products will be analyzed. Transient and transgenic knock-out/down and overexpression approaches will be employed to determine the authentic roles of the studied genes in plant defense mechanism. Target genes of selected ERFs will be identified by taking transcriptomic approaches. Furthermore, as the nature of BW shares commonness with that of water

deficit (WD), roles of the studied ERFs in plant responses to drought and salinity will be investigated as well through coordination with other sub-projects. Additionally, with the well-established BW-bioassay systems, this sub-project could provide assistance to determine whether the genes proposed to be studied in other sub-projects involve in plant response to BW. Through the integration of this Programmatic Project, the functional genomic studies proposed here are expected to gather important information about plant defense mechanisms against important diseases and water deficit, as well as to assemble resources useful for crop biotechnology.

計畫編號：BM05-06
計畫名稱：植物耐熱基因的分離和研究
計畫主持人：鄭石通
計畫摘要(中)：

根據美國國家海洋大氣署的調查，自十九世紀末期以來，地表溫度已上升了 0.6℃，而過去的二十五年來溫度上升最為嚴重，地表溫度上升了 0.2~0.3℃。聯合國也表示地表平均溫度到 2100 年則可能再增加 3.5℃。1981 至 2002 年間，全球暖化已經造成小麥、玉米和大麥等作物每年四百萬噸的損失，估計每年損失 50 億美元。然而大多數研究高溫對於植物的影響，都專注於植物營養組織的熱休克誘導的部分。然而前人研究指出隨著溫度的上升，植物生殖組織，如未來會發育成種子或果實的雌蕊及雄蕊，對於溫度敏感性較營養組織來的大。因此在到達熱休克反應的溫度之前，溫度的增加就已經損害了作物的產量。因此，本計劃將研究中度溫度對於植物生殖組織的影響。本計劃將利用在正常溫度(22℃)及中度溫度(32℃)下發育的阿拉伯芥花軸組織做為材料，以其 cDNA 作為探針與阿拉伯芥之生物晶片進行雜交。由生物晶片的結果可以得到會受到熱干擾的基因，這些基因包括了受熱促進及受熱降低表現的基因，本實驗室會再以 RT-PCR 進一步的確認其表現和溫度的相關性。同時會以 Blastn 及 Genevastigator 比對和分析程式，對這些基因的功能和其所參與的訊息傳遞途徑加以分析。接著會以 35S 或組織專一性的啟動子在阿拉伯芥中大量表現這些基因。同時也會利用 RNAi 產生 knock-down 轉殖株，並向阿拉伯芥生物資源中心(ABRC)購買利用 T-DNA 插入所產生的 knock-out 植株，這些轉殖研究將可以充分了解，這些能受到熱干擾的基因在植物

體中的功能。這些在阿拉伯芥中所進行研究的基因，如果產生有趣的結果，將進一步轉殖進入蕃茄中，並且以花粉的活性及著果率來測試轉殖蕃茄對熱的容忍度。本實驗室也會對這些受熱干擾基因的啟動子作系列性的刪減，且將這些啟動子下游接上報告基因，並觀察溫度對於報告基因在原生質體中和植物體中表現的影響。實驗也將會分析訊息傳遞物質，如鈣離子、超氧歧化物、蛋白質激酶和蛋白質磷酸水解酶等的表現，希望能藉此了解植物中受熱誘導的訊息傳遞途徑。期待本計劃的實驗結果可以使我們了解植物中，高溫干擾基因的功能及其所參與的訊息傳遞路徑，並且在全球暖化的時期，能促進番茄和其他農作物的產量。

計畫摘要(英)：

Global surface temperatures have increased about 0.6˚C since the late-19th century, and about 0.2 to 0.3˚C over the past 25 years, the period with the most credible data reported by National Oceanic and Atmospheric Administration of US. Also, the United Nations on climate change also mentioned that Earth's average surface temperature could rise up to 3.5˚C by 2100. Global warming has already reduced the combined production of wheat, corn, and barley by 40 million metric tons per year between 1981-2002, and the annual losses was estimated to be $5 billion. However, most of studies about temperature in plants focus on the effects of high temperature shock, so called heat shock, on the vegetative tissues of plants. Previous studies indicated that reproductive tissues, containing stamen and carpel that further develop to fruits, seeds and grains, are more sensitive to temperature rise than vegetative tissues. The increase of temperature impairs the productivity of crops, before it goes up to the temperature high enough to induce heat shock response. Therefore, this project is planned to study the effects of moderate temperature, not heat shock temperature, on plant reproductive tissues. First, the difference of gene expression patterns of Arabidopsis growing in 22℃ (normal temperature) and 32℃ (moderate temperature) is studied with DNA microarray using cDNA from inflorescence tissues as probes. The possible thermo-interference genes, including thermo-enhancement and thermo-reduction genes, will be identified by RT-PCR, and their putative functions and signal transduction pathways will be analyzed by Blastn

and Genevestigator programs. Transgenic Arabidopsis over-producing these heat-interference genes will be produced using 35S promoter or tissue-specific promoter, and those with knocking-down and knocking-out these heat-interference genes will also be generated by creating RNAi plants or requesting T-DNA insertion lines from Arabidopsis Biological Resource Center (ABRC). These researches results from transgenic plants may reveal the functions of these thermo-interference gene in details. The interesting genes from Arabidopsis studies may also be transferred into tomato, and the thermotolerance of transgenic tomato will be examined by pollen viability and fruit set. Promoters of thermo-interference genes will also be studied by series deletion, and after promoter fused to reporter genes its expression under the effects of temperature will be investigated. Further, signal messengers, such as calcium ion, reactive oxygen species (ROS), protein kinase, and protein phosphatase will be analyzed to elucidate the signal transduction pathway of thermo-induction in plants. The results of this study may understand the functions and signal transduction pathway of thermo-interference genes in plants, and enhance the yields of tomato and other crops in the global warming period.

計畫編號：BM05-07

計畫名稱:鈣離子螯合劑 EGTA 破壞植物獲得耐熱性之生理功能研究

計畫主持人：靳宗洛

計畫摘要(中)：

目前已知在非致死高溫的處理下，熱休克蛋白（heat-shock protein）可保護其他蛋白質分子，免於受到溫度逆境的影響。本實驗室於先前研究中發現，黃豆（Glycine max）和水稻（Oryza sativa）的白化幼苗，在外加鈣離子螯合劑（EGTA）並以高溫處理的情況下，會喪失獲得性耐熱能力，並使胞內滲透更加嚴重。此時若另外添加二價陽離子（特別是鈣離子）即可恢復對此溫度的耐受性。EGTA 影響植物對於高溫耐受性之生理機制及其訊息傳導路徑為本研究室將來的研究重點。我們將深入探討在此過程之中是否有 sHSP 之參與，或是在 sHSP 外之另一個調控機制。此外，額外添加鈣離子螯合劑、L 型鈣離子通道抑制劑，以及調鈣素(CaM)拮抗劑，對高溫處理下細胞內鈣離子濃度提高能力之影響也為研究重點之一。我們也將研究此種

與 EGTA 結合、游離狀態的細胞間鈣離子，是否參與中膠層中鈣離子-pectate 複合體之形成，並且對於高溫狀態下，細胞壁維持細胞膜完整性以及訊息傳遞有關。另外我們也將研究在高溫環境中，細胞間鈣離子和調鈣素之交互作用，以及此種作用對於水稻內的熱休克蛋白基因表現的影響。我們期望釐清 EGTA、細胞間鈣離子和水稻內調鈣素的交互作用，以期能更了解其對於植物耐熱性所扮演的角色。

計畫摘要(英)：

The effects of heat-shock protein (HSP) induced by non-lethal HS treatment, which act as molecular protein chaperones to confer thermotolerance, have been widely reported. In our preliminary experiment, we abolished the acquired thermotolerance of etiolated soybean (Glycine max) and rice (Oryza sativa) seedlings by an exogenously supplied Ca2+ chelator, EGTA, and this might related to increased cellular content leakage during HS treatment. Thermotolerance was restored by the addition of divalent cations, specifically Ca2+, during EGTA incubation. This suggested Ca2+ was involved in the acquisition of thermotolerance. Whether this effect was dependent or independent on sHSP expression and function is interesting and deserve for further studying. To specify the role of Ca2+ in thermotolerance, the oscillation of cytoplasmic Ca2+ level ([Ca2+]cyt) during HS treatment will also be observed. Why the extracellular EGTA removable Ca2+ during HS response is important to confer thermotolerance, what is the physiological function, and how to transmit signal to induce HS response are the topics that we are interested in. In this proposal, we focus on the leaked reabsorbable Ca2+ which disallowed by EGTA, to test the possibility for it to participate in Ca2+-pectate formation in the middle lamella. The importance of cell wall remodeling to retain plasma membrane integrity and for coordination with HS signaling in acquisition of thermotolerance needs to be clarified. Whether the apoplastic HS released/reabsorbed Ca2+ interacts with CaM to modulate Ca2+ influx, and whether this increased [Ca2+]cyt transients might control OsHSP genes expression will also be analyzed. We want to show the significance of intercellular Ca2+ as well as Ca2+/OsCaM mediating HS signaling to confer thermotolerance in planta.