称号及び氏名	博士(応用生命科学)　白柿　薫平
学位授与の日付	2021年3月31日
論　文　名	Molecular biological and evolutionary analysis of hybrid weakness in *Capsicum annuum* × *Capsicum chinense* （*Capsicum annuum* × *Capsicum chinense* におけるトウガラシ雑種弱勢に関する分子生物学的および進化学的研究）
論文審査委員	主査　横井　修司 副査　高野　順平 副査　東條　元昭 副査　手塚　孝弘

論文要旨

Hybrid weakness, a type of postzygotic isolation in reproductive isolation, is defined as abnormal growth in F_1 plants derived from crosses between two normal parents. This phenomenon often prevents plant breeding programs due to difficulty in obtaining offspring. Moreover, it is thought that hybrid weakness contributes to speciation. Therefore, the study of hybrid weakness is important in academic and industrial fields. However, the mechanism of hybrid weakness is not yet fully understood, even though it has been extensively studied in recent years.

This study aimed to investigate the mechanism of hybrid weakness in the cross of *Capsicum annuum* (chile or sweet pepper) × *Capsicum chinense* (bonnet pepper). The hybrid weakness is caused by two complementary dominant genes, and it is reported that *C. annuum* can possess the *aaBB* genotype while *C. chinense* and *C. frutescens* can possess the *AAbb* genotype (Inai et al. 1993, Yazawa et al. 1989). Despite these previous studies, however, the research on *Capsicum* hybrid weakness had not been advanced any further. In Chapter 1, I investigated the morphological and physiological characters of *Capsicum* F_1 hybrids showing hybrid weakness. In Chapter 2, I investigated when hybrid weakness was induced in the plant growth phase. In Chapter 3, I focused on the changing time course after the induction of hybrid weakness. In Chapters 4 and 5, I studied about the relationship between hybrid weakness and speciation in *Capsicum*.

Chapter 1. Characterization of *Capsicum* hybrid weakness

I characterized morphologically and physiologically hybrid weakness in *Capsicum*. F_1 hybrids did not show weaker growth than their parents after 20 days after germination (DAG), but at 40 DAG, the hybrid weakness phenotype was evidenced by almost complete arrest of new leaf formation, delayed increase in plant height, and reduced upper internode length. The shoot apical meristem (SAM) of F_1 hybrids exhibited delayed development and an abnormal structure characterized by a flat shape and the presence of fuzzy cell layers on the surface. These abnormal SAMs of F_1 plants may lead to dwarfism. Dead cells and accumulation of H_2O_2, one of the reactive oxygen species, were visually detected in leaves of F_1 hybrids, and cell death was considered to be programmed, as it was accompanied by internucleosomal fragmentation of DNA. The expression of immunity marker genes *PR1* and *PR2* was upregulated in leaves of F_1 hybrids. These results suggest that a hypersensitive response-like reaction is involved in *Capsicum* hybrid weakness.

Chapter 2. Abnormalities in juvenile-to-adult transition in *Capsicum* hybrid weakness

I surveyed morphological traits that are reported to change during juvenile-to-adult (JA) transition in vegetable phase in several plant species using the first to 9th leaves at 60 DAG. Length / width ratio, and presence or absence of trichome in the leaves didn’t almost change by leaf position in *Capsicum*. Petiole / leaf blade ratio decreased as leaf position increased. The decrease of petiole / leaf blade ratio reached plateau at 5th, 6th, and 7th leaves position in respectively *C. annuum*, *C. chinense*, and F_1 hybrids. The number of lateral veins increased as leaf position increased in both parents and F_1 hybrids, and those in F_1 hybrids were less than those in both parents. In toluidine blue staining, the color was pink until 5th leaves, and it was blue from 7th leaves in both parents, but the color didn't change in leaves of F_1 hybrids. The number of stomata increased as leaf position increased, and rapidly increased from 6th leaves at both parents. However, the increase kept moderate in F_1 hybrids, and the number of stomata in F_1 hybrids was less than that of both parents from 6th leaves. Based on these morphological surveys, I estimated that juvenile phase was until 5th leaves, and adult phase was from 7th leaves in both parents. On the other hand, the JA transition was delayed or not progressed in F_1 hybrids. The phenotype of hybrid weakness appeared when the 5th leaves almost fully expanded. Therefore, hybrid weakness is suggested to be induced during JA phase transition.

Chapter 3. The time course of temperature-dependent *Capsicum* hybrid weakness in gene-expressional, physiological and morphological changes

I showed that *Capsicum* hybrid weakness was suppressed at 30 and 35°C, and was induced at 15, 20, and 25°C. Moreover, I investigated the time course of hybrid weakness in cell death, metabolite content, and gene expression in leaves of plants transferred to 20°C after growing at 30°C for 21 days. The expression of pathogen defense-related genes was upregulated 1 day after transfer to 20°C (DAT). Cell death was detected at 7 DAT, plant growth had almost stopped since 14 DAT, and sugars were accumulated at 42 DAT in hybrid plants. The study revealed that some sugar transporter genes, which had been upregulated since 7 DAT, were involved in sugar accumulation in *Capsicum* hybrid weakness. Thus, these results demonstrated that gene expression changes occur first, followed by physiological and morphological changes after induction of hybrid weakness. The responses observed in this study in *Capsicum* hybrid weakness are likely to be owed to plant defense responses-like reactions.

Chapter 4. Phylogenetic analysis of *Capsicum* based on rDNA-ITS region

The rDNA-ITS sequences and morphological traits of domesticated and wild *Capsicum* species were examined. The accessions of *C. annuum*, *C. chinense* and *C. frutescens* were closely related according to morphology characters because no trait can distinguish one another. The phylogenetic tree based on rDNA-ITS formed the *C. annuum* clade, the *C. chinense* and *C. frutescens* clade, the *C. baccatum* clade, and the *C. pubescens* clade. *C. annuum*, *C. chinense* and *C. frutescens*, which may have *A* or *B* allele for hybrid weakness, were also closely related in the phylogenetic tree; especially, *C. chinense* and *C. frutescens* were genetically very close. *C. annuum* accessions having *b* allele belonged to one clade in the *C. annuum* clade. It was suggested that *Capsicum* hybrid weakness could be involved in speciation of *C. annuum*.

Chapter 5. Phylogeographic analysis of *Capsicum* hybrid weakness

I surveyed whether 63 *C. annuum* accessions had *B* or *b* allele for hybrid weakness by test crossing with *C. chinense* having *A* allele. Out of the 63 *C. annuum* accessions, five accessions had *B* allele; three accessions were native to Latin America, and two accessions were native to Asia. I calculated the percentage of geographic distribution of the *B* carriers of *C. annuum* by Latin America, Europe, Africa, and Asia, based on the results of the present and previous studies (Yazawa et al. 1989). The percentage of *B* carriers was 42% in Japan, 13 % in Asia excluding Japan, 8% in Latin America, and 0% in Europe and Africa. Additionally, the 48 accessions of *C. annuum* from various countries were subjected to SSR analysis. The number of the clade with high percentages of *B* carriers was two in UPGMA tree, and was one in NJ tree. In the graph by principal coordinate analysis, most *B* carriers were localized in a single group although the group also included *b* carriers. I presumed that the *B* allele was acquired in some *C. annuum* lines in Latin America, and both *B*-carrying and *b*-carrying lines were introduced to the world during the Age of Discovery.

I discussed the mechanism of *Capsicum* hybrid weakness based on the results of my research. *Capsicum* hybrid weakness was triggered by beginning JA transition, suggesting that a factor expressed during JA transition may affect the causal genes of hybrid weakness. Then, the interaction of two causal genes induced auto-immune responses accompanied with reaction oxygen species, cell death, and upregulation of genes of PR proteins and sugar transporters. Causal genes have not yet identified, but I guessed that one or two of the causal genes are the leucine-rich repeat receptor-like protein kinase genes as reported in hybrid weakness in other species, because auto-immune response occurs in *Capsicum* hybrid weakness. Moreover, I presumed that activation of sugar transporters by auto-immune responses may prevent sugar translocation, which plays an important role in JA transition. That may be why F_1 hybrids cannot completely transit to adult phase in vegetable phase. Besides, I suggested the possibility that *Capsicum* hybrid weakness is involved in speciation of *C. annuum*, *C. chinense* and *C. frutescens*.

審査結果の要旨

異なる生物集団間で交配したときに、後代が得られない現象を生殖的隔離機構という。この現象は集団間において独立に進化した遺伝子が不適合を示すことで生じるとされている。本現象は種分化において重要な働きを担う一方で、新たな品種創出のために遠縁交雑育種を行う際には大きな障害となっており、その障壁を取り除くことが求められている。したがって生殖的隔離機構の研究は、進化のメカニズムの解明といった学術的な側面に加え、育種などの産業的な側面からも重要な研究課題である。

生殖的隔離機構は、受精前隔離と受精後隔離に二分される。さらに受精後隔離には、種子発達不全、雑種致死、雑種弱勢、雑種不稔、雑種崩壊など多くの現象が含まれる。しかし、これらの現象の仕組みは十分に解明されていないのが現状である。本研究では、*Capsicum*（トウガラシ）属の *C. annuum* × *C. chinense* における雑種弱勢について解析を行った。主に、分子生物学的手法を用いて分子メカニズムの解明、ならびに系統地理学的手法を用いて雑種弱勢現象の獲得過程の解明を目的とした。

第 1 章では、トウガラシ雑種弱勢の形態的および生理的な特徴を明らかにした。F_1 植物の生育は発芽後 30 日目頃に鈍化し始め、40 日目頃にはほぼ停止した。茎頂分裂組織の組織学的観察によって、F_1 植物では茎頂の分裂活性が低いことが示唆された。F_1 植物と両親の葉を用いて生理的な解析をすると、F_1 植物でのみ、細胞死と過酸化水素が検出された。さらに、細胞死がプログラムされた細胞死であることや病害応答マーカー遺伝子が F_1 植物で特異的に発現することも示された。これらの結果より、トウガラシ雑種弱勢に自己免疫応答が関与することが示唆された。

第 2 章では、栄養生長相を構成する幼若相から成熟相への相転換に注目してトウガラシ雑種弱勢を解析した。葉の形態的特徴によって、F_1 植物と両親の相転換が開始されるタイミングが第 5 葉期であることが示唆された。両親が成熟相へ完全移行するタイミングは第 7 葉期であったが、F_1 植物は完全移行しなかった。トウガラシ雑種弱勢の表現型が現れるタイミングは第 5 葉が完全展開するタイミングと一致しており、成熟相への転換開始が弱勢誘導の条件であることが示唆された。さらに、弱勢が発現することによって成熟相への完全移行が妨げられる可能性も示された。

第 3 章では、トウガラシ雑種弱勢が高温条件で抑制されることを明らかにした。さらに、高温抑制を利用して雑種弱勢を誘導するタイミングを同期させる実験系を確立し、雑種弱勢のタイムコースを調査した。弱勢誘導後 7 日目に病害抵抗性関連遺伝子およびショ糖トランスポーター遺伝子の発現上昇、ならびに細胞死が検出された。そして、14 日目に植物体の生育が停止し、42 日目には糖およびクエン酸が蓄積していることを明らかにした。

第 4 章では、トウガラシ栽培種 5 種を中心とした系統を対象とし、rDNA-ITS 領域の配列による系統解析を行った。トウガラシ雑種弱勢に関与する *C. annuum*、 *C.*

chinense、*C. frutescens* は互いに近縁であることが示され、雑種弱勢の原因遺伝子を持たない *C. annuum* の系統どうしが遺伝的に近縁である傾向が明らかとなった。さらに、雑種弱勢原因遺伝子を持つ、もしくは持たない *C. annuum* 系統の遺伝的関係に関する大規模な解析を行うために、第 5 章では *C. annuum* の多数系統を用いて、雑種弱勢原因遺伝子に注目して系統地理学的解析を実施した。その結果、*C. annuum* が持つ雑種弱勢原因遺伝子は中南米で獲得され、大航海時代以降に、雑種弱勢原因遺伝子を持つ系統と持たない系統が同時に全世界に拡散され、土着の品種となったことが示唆された。

以上のように本研究では、トウガラシ雑種弱勢の分子メカニズムおよび雑種弱勢原因遺伝子獲得の史実に関する知見が得られた。これらの知見は、植物の生殖隔離機構において新しい理解を与え、その克服法開発に寄与するなど、植物育種学において貢献するものである。よって、本論文の審査ならびに最終試験の結果と併せて、博士（応用生命科学）の学位を授与することを適当と認める。