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Review

Nucleus-cytoplasm compatibility: A genetic system underlying allopolyploid speciation as exemplified in alloplasmic lines of wheat

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Summary In the tribe Triticeae, systematic studies using alloplasmic lines have greatly facilitated our understanding of cytoplasmic genome inheritance, its structural and functional diversity, and interaction with nuclear genomes. Effective utilization of these valuable experimental tools produced by combining given nuclear genomes with cytoplasmic genomes from various related species has provided ample evidence supporting the presence and roles of a genetic system controlling nucleus-cytoplasm compatibility operating particularly in the lineages of allopolyploid speciation. This review focused on the current state of our knowledge of the nucleus-cytoplasm compatibility system. It also emphasized the necessity for further research toward more precise identification and understanding of its complex genetic regulatory mechanism and possible practical application in agriculture.

Keywords Nucleus-cytoplasm compatibility, Alloplasmic line, Allopolyploid speciation, Heteroplasmy, Triticeae, Wheat.

Eukaryotic organisms have evolved through endosymbiosis. Consequently, plant genomes are compartmentalized in the nucleus and the two cytoplasmic organella, *i.e.*, chloroplasts functioning in photosynthetic carbon assimilation and mitochondria functioning in oxidative phosphorylation. Due to this endosymbiotic origin, cytoplasmic genomes or plasmons have coevolved through complex interactions with each other and with nuclear genomes to attain nucleus-cytoplasm (hereafter abbreviated as NC) compatibility, which is required to ensure proper cellular function throughout the whole developmental stages of plants (Sloan *et al.* 2018, Bogdanova 2020, Postel and Touzet 2020).

In contrast to the biparental mode of inheritance of nuclear genomes, cytoplasmic genomes display uniparental inheritance (maternal in general) during fertilization and development. Genetic analysis of cytoplasmic genomes has long been hampered because of the lack of methodology to create heterozygosity due to their uniparental inheritance. However, rather than taking advantage of this, cytoplasmic genomes of related species could be experimentally combined with specific nuclear genomes through substitution backcrosses of F_1 hybrids by recurrent nuclear parents. The caveat is that repeated backcrosses may not necessarily result in complete genome substitution of nuclear genomes at the nucleotide level due to the presence of remnant parental genomes, and the introduced nucleotide polymorphisms during backcrosses might have phenotypic effects on resultant alloplasmic lines (Matsuoka *et al.* 2014, Banouh *et al.* 2023). Polymorphisms due to various mechanisms, including heteroplasmy, also occur in the cytoplasmic genomes (Noyszewski *et al.* 2014).

Adopting this methodology, Kihara (1951) produced alloplasmic hybrids of wheat (*Triticum aestivum*) and showed that the cytoplasm of *Ae. markgrafii* (Greuter) Hummer (synonymous with *Ae. caudata*) induced male sterility. This was the first demonstration of specific interaction expressed between nuclear and cytoplasmic genomes in the Gramineae. Following this pioneering work, Prof. K. Tsunewaki (1980, 1993, 1996), taking leadership in close collaboration with domestic and international researchers, conducted systematic studies to produce a complete set of alloplasmic lines, in which 12 different nuclear genomes of hexaploid wheat were combined with cytoplasmic genomes of 47 different accessions from 32 species covering all diversity in the *Triticum-Aegilops* complex. Based on wide phenotypic spectrums displayed in these alloplasmic lines, all cytoplasmic genomes in the complex have been classified

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into 18 major types plus 5 subtypes, and their evolutional lineages together with those of nuclear genomes clarified (Tsunewaki 2009, Tsunewaki *et al.* 2002).

NC compatibility exemplified in Triticeae

Allopolyploidy resulting from hybridization followed by hybrid genome doubling (amphidiploidization) of divergently related species in Triticeae (Kihara 1954, Feldman and Levy 2012) often have encountered severe disruption of cellular function due to conflicts through genetic and ecological mechanisms (Matsuoka *et al.* 2014) and perhaps also between interacting nuclear and cytoplasmic genomes during its evolution. NC compatibility is known to be the system that ensures functional coordination between these interacting genomes in allopolyploid lineages (Grover *et al.* 2022). Herein representative studies are reviewed focusing on specific NC compatibility that controls seed viability and plant vigor often associated with the regulation of male fertility and sterility in NC hybrids.

Nuclear genomes of hexaploid or common wheat are incompatible with the cytoplasm of *Triticum monococcum* ssp. *boeoticum* and thus NC hybrids with this cytoplasm exhibit severe growth depression and male sterility (Tsunewaki 1980). To characterize these NC hybrids, a comparative analysis was conducted on photosynthetic and respiratory activities among 19 hybrids with different cytoplasms from *Triticum* and *Aegilops* species (Nakamura *et al.* 1991a). The study showed that this cytoplasm was unique causing abnormally high carbon assimilation rate and cytochrome c oxidase (COX) activity. This apparently negative correlation of the cytoplasmic organellar activities with the reduced plant vigor and male sterility was suggested to be due to NC incompatibility specifically induced in the hybrids.

All nuclear genomes of hexaploid wheat are compatible with the cytoplasmic genome of *Ae. tauschii* (synonymous with *Ae. squarrosa*) because of their possession of D genome (Tsuji and Murata 1976). On the other hand, tetraploid wheat (*T. durum* or *T. turgidum*) hybrids with this cytoplasm produced shriveled abortive seeds, while plants raised after embryo rescue exhibited reduced plant vigor and male sterility. Ohtsuka (1981, 1991) conducted extensive studies on tetraploid wheat hybrids with this cytoplasm to show that nuclear genomes of more than half of durum wheat are incompatible, while all except for one of wild and cultivated timopheevii wheat (*T. araraticum* and *T. timopheevii*) are compatible. Seed plumpness with normal germinability, plant vigor, and male fertility were fully restored when a nuclear gene *Ncc-sqr1D* located on chromosome 1D was present. *T. timopheevii* possesses *Ncc-tmp1A*, which is likely homoeologous to *Ncc-sqr1D* and confers full compatibility on the hybrid with *Ae. tauschii* cytoplasm when it is introgressed into a nuclear genome of *T. durum* (Asakura *et al.* 1997a, b, 2000). Southern blot analysis using restriction fragment length polymorphism (RFLP) markers on the homoeologous group 1 chromosomes could locate *Ncc-tmp1A* in the centromeric region of chromosome 1A of *T. timophheevii*. Segregation analysis of seed viability (seed plumpness) and endosperm γ-gliadin composition in test crosses involving two types of NC hybrids (*Ae. tauschii*)–*T. timopheevii* and (*Ae. tauschii*)–*T. turgidum*, showed that G genome also possesses a functional homoeoallele *Nccp-tmp1G* on chromosome 1G. Two sequence-tagged site markers closely linked to *Ncc-tmp1A* were detected in all accessions of *T. urartu* but not in *T. boeoticum* analyzed. The result suggested the conservation of genome sequences adjacent to *Ncc-tmp1A* in *T. urartu*, an A genome donor for emmer and timopheevii wheat, and its characteristic differentiation in the two groups of tetraploid wheat (Asakura *et al.* 2001).

Similar NC incompatibilities associated with reduced plant vigor and male sterility were reported in *T. turgidum* with the cytoplasm either of *Ae. cylindrica*, *Ae. uniaristata* or *Ae. longissima*. In these hybrids, two different nuclear genes could restore NC compatibility, *i.e.*, *scs* derived from *T. timopheevii* and *Vi* probably arose by a spontaneous mutation in hybrids with *Ae. cylindrica* cytoplasm (Maan 1992, Anderson and Maan 1995). Aneuploid analysis using RFLP markers together with segregation analysis of seedling vigor (indicating the presence of *scs*) and selfed-fertility (indicating the presence of *Vi*) revealed that *scs* is located on the long arm of chromosome 1A and *Vi* on the short arm of chromosome 1B. Homoeoallelic genes such as scs^{ti} and scs^{ae} have been identified and localized respectively on chromosome 1A of *T. timopheevii* and 1D of *T. aestivum* (Hossain *et al.* 2004). Gehlhar *et al.* (2005) further identified a homoeologous gene scs^{d}, selected from compatible durum wheat producing all viable plump seeds and fertile plants when crossed to NC hybrids with *T. longissima* cytoplasm.

Chromosome 1R of rye (*Secale cereale*) or a midget chromosome representing the pericentric centromere region of the long arm of chromosome 1R was necessary for the development of plump and viable seeds and restoration of male fertility in hexaploid wheat with the rye cytoplasm (Murata *et al.* 1992). Similar NC compatibility was found to control plant vigor and male fertility in hexaploid wheat with the cytoplasm of *Agropyron* (wheatgrass) species, either *Ag. trichophorum* (synonym of *Ag. intermedium* ssp. *barbulatum*) or *Ag. glaucum* (*Ag. intermedium* ssp. *intermedioum*) (Tsujimoto *et al.* 1987, Nakamura *et al.* 1991b, Suzuki *et al.* 1992). These NC hybrids exhibited severely depressed vigor and complete male sterility, while almost normal plant vigor and male fertility were restored when the centromeric region of the short-arm of homoeologous group 1 chromosome derived from the maternal *Agropyron* was present. Studies on photosynthetic carbon assimilation and oxygen uptake and evolution showed that observed reduced rates of photosynthesis in the depressed line were due to increased rates of dark respiration in green leaves (Suzuki *et al.* 1994). The observed growth depression and male sterility in the NC hybrids were shown to be associated with markedly elevated levels of mitochondrial transcription activity measured by run-on transcription analysis as compared to the normal line (Suzuki *et al.* 1995), suggesting that NC incompatibility is related to the aberrant mitochondrial transcription. Another NC hybrids of wheat with the cytoplasm of either *Elymus trachycaulus* or *E. ciliaris* exhibited male sterility and reduced plant vigor, but the addition of homoeologous group 1 chromosome derived from the cytoplasm donors could restore normal male fertility and plant vigor (Jiang *et al.* 1992).

Mapping and molecular identification of Ncc genes

The first step needed to understand the genetic control of NC compatibility is to map and identify responsible genes. As described above, several *Ncc* genes responsible for conferring NC compatibility have been identified and localized on the group 1 chromosomes in Triticeae. The detailed mapping of *Ncc* gene was reported for scs^{ae} (Simons *et al.* 2003). Analyses using markers, including RFLP, amplified fragment length polymorphism (AFLP), and microsatellite, were performed to map scs^{ae} in a region of chromosome 1D delineated by two flanking marker loci at distances of 0.6 to 2.3 cM. The gene *scs^{ae}* was further mapped within a region of 12.9 centi ray by means of *in vivo* radiation hybrid (RH) mapping (Michalak de Jimenez *et al.* 2013). Bassi *et al.* (2016) developed a novel *in silico* approach to identify open reading frames (ORFs) of the gene *scs^{ae}*. Additional markers were developed to anchor short contigs of ORFs to the RH map, and the region containing the *scs^{ae}* locus was narrowed down to a single contig of the bacterial artificial chromosome of *Ae. tauschii*. Sequencing and assembly by nano-mapping further allowed the identification of a gene *rhomboid* as the only ORF existing within the refined *scs^{ae}* locus. The exploitation of novel technologies such as nano-mapping and *ad hoc* algorithms made it possible to achieve 'gene-landing' to a single candidate gene belonging to members of the *rhomboid* gene family, which encode intermembrane proteases. Furthermore, a single nucleotide mutation in this gene was shown to create observed variations controlling NC compatibility. Further experiments involving transformation and/or gene editing are needed for functional confirmation of this *rhomboid* gene as one *Ncc* gene.

Heteroplasmy and its control by NC compatibility

Heteroplasmy, *i.e.*, the presence of a mixture of maternal and paternal organellar genomes, has frequently been observed in plants, particularly in allopolyploids. Mitochondrial heteroplasmy was detected in 6 out of 15 genomic regions studied by RFLP analysis in tetraploid and hexaploid wheat with D or D^2 cytoplasms (Tsukamoto *et al.* 2000). Analysis of polymerase chain reaction (PCR)-RFLP showed that a polycistronic transcriptional unit *nad3-orf156* contained not only the maternal but also the paternal and novel-paternal-like sequences. Relative stoichiometry of these polymorphic sequences varied depending on the cytoplasms and the nuclear backgrounds, suggesting that the differential amplification of the heteroplasmic copies is under the control of NC compatibility. Hattori *et al.* (2002) mapped all polymorphic sites in the *nad3-orf156* region detected in the NC hybrids and parental lines by comparative RFLP analysis and sequencing of random PCR clones. More maternal copies were present in hybrids with D cytoplasm, whereas more paternal copies were in hybrids with D^2 cytoplasm. All editing sites and their editing frequencies were conserved among the hybrids, and only the maternal pattern of editing was detected. Structural heterogeneity present in the *nad3-orf156* region in NC hybrids of *T. timopheevii* with the compatible D cytoplasm of *Ae. tauschii* was further studied and compared with the parental lines (Kitagawa *et al.* 2003). The NC hybrids possessed all five sequence types identical to those found in the paternal *T. timopheevii* parent in about 30% relative stoichiometry, while the remaining 70% comprised only one of two maternal sequence types. No paternal-identical or -related sequences were present in the D plasmon. These results provided experimental evidence for the paternal transmission of the mitochondrial DNA and moderately selective NC interaction to amplify the paternal copies.

Kitagawa *et al.* (2003) compared transcript profile and post-transcriptional processing *orf156* among tetraploid and hexaploid wheat NC hybrids with D cytoplasm and compared them with their parental lines. The wheat *orf156* is transcribed as a polycistronic transcriptional unit of *nad3-orf156*, and the precursor mRNA is post-transcriptionally cleaved. Despite heteroplasmic copies in the hybrids, only single maternal-type transcripts were detected and edited among reverse transcription PCR products of *orf156* mRNA. Likewise, in the parental euplasmic lines, only major maternal copies were transcribed and edited. However, RNA gel blot analysis using an orf156 probe showed the presence of different-sized transcripts in the different tissues in different lines. Primer extension analysis revealed the presence of different 5′ termini of *orf156* transcripts, which most probably represent alternative post-transcriptional cleavages of the precursor mRNA. These cleaved transcripts were polymorphic, but the paternal-like cleavages predominated in the hybrids, irrespective of the tissues studied. The results suggested that both preferential transcription of the maternal copies and paternal-specific post-transcriptional cleavages are controlled by nuclear gene(s) involved in the NC compatibility system.

Similar heteroplasmic conditions were reported by Kawaura *et al.* (2011) in three mitochondrial genes, *atp4, atp6, and rps7*, in alloplasmic wheat cv. Chinese Spring (CS) with the cytoplasm of *Ae. crassa* 6*x*. Based on cloned and sequenced PCR products, relative stoichiometries of the different mitochondrial gene types were compared among the NC hybrids and F_1 hybrids of *Ae. crassa* 6*x* pollinated by the nuclear donor as well as their parental lines. They found the presence of CS-type copies in the NC hybrids and *Ae. crassa* 6*x* together with *Ae. crassa* 6*x*-type copies in CS. Varying levels of heteroplasmic copies were present in all lines studied, and comparisons between the F_1 and the NC hybrids showed that frequencies of the paternal copies increased during successive backcrosses. Transcript analysis, however, showed that only maternal transcripts were present and that expression of minor paternal heteroplasmic copies was silenced.

In NC hybrids of wheat with barley (*Hordeum vulgare*) cytoplasm, varying levels of heteroplasmic states were detected in several genomic regions at early backcross generations (Aksyonova *et al.* 2005). NC hybrids characterized by male sterility and reduced plant vigor showed homoplasmy of the maternal-type copies, whereas fertile and vigorous hybrids showed homoplasmy of the paternal-type copies. It was thus suggested that the observed paternally-oriented shift of organellar DNA composition or breakdown of the heteroplasmic state was correlated with the restoration of male fertility and plant vigor. Recombinant NC hybrids of (*H. vulgare*)-*T. aestivum* showing varying levels of fertility and plant vigor were further selected after backcrosses by various wheat accessions, and a possible association of heteroplasmy and NC compatibility was studied. After monitoring the composition of four mitochondrial and two chloroplast genome regions in male sterile F_1 NC hybrids and backcrossed progenies derived from the vigorless and male sterile F_1 and BC_1 with several different wheat parents, they detected different levels of heteroplasmic states prevailed in mitochondrial *cox1*, a 5′-flanking region of *cob*, *nad3*-*orf156* and a 5′-upstream region of 18S/5S repeats. In contrast, only maternal copies were detected in a simple sequence repeat (SSR) loci downstream of *trnS* and a 3′-flanking region of *rbcL* of the chloroplast genome. In vigorous and fertile plants possessing recombinant nuclear genomes, however, only paternal wheat copies of both organellar DNA were detected. The results suggested that transmission followed by selective amplification of the paternal wheat organellar DNA led to the paternally-oriented shift, which was apparently correlated with the restoration of plant vigor and male fertility during the successive backcrosses. It was further shown that chromosome 1BS of wheat was responsible for the restoration of male fertility in these recombinant NC lines (Truvacheeva *et al.* 2021).

It is well known that many organellar proteins comprise multi-subunits that are encoded by both nuclear and cytoplasmic genomes. NC conflict arises to result in biased transcription of the maternally derived nuclear progenitor genomes in allopolyploid wheat (Grover *et al.* 2022). Coevolutionary processes of nuclear *rbcS* genes encoding small subunits of Rubisco and plastid translocons, which mediate recognition and translocation of nuclear encoded proteins into chloroplasts, were studied in *Triticum* and *Aegilops* species (Li *et al.* 2020). They demonstrated that intergenomic paternal-to-maternal gene conversion occurred in the homoeologous *RbcS3* gene from the D-genome progenitor *Ae. tauschii*, and thus NC selection of the maternal-type or B-subgenome-type *RbcS3*-encoded small subunit prevails in hexaploid wheat but not tetraploid wheat. The results imply that early selection favors targeting the maternal-type subunits in hexaploid wheat and that NC coevolution is mediated by organellar targeting and transportation of nuclear-encoded proteins.

A similar situation was reported in a male-sterility gene of hexaploid wheat. Among five genic male sterility genes identified in hexaploid wheat, a recessive mutant allele at a locus *MS1* was cloned by advanced map-based cloning (Wang *et al.* 2017). MS1 protein was shown to be a phospholipid-binding protein localized to plastid and mitochondrial membranes. Notably, it was found that only B genome-homoeolog of this gene was expressed. Still, other homoeologs from incompatible A and D genomes were epigenetically silenced in hexaploid wheat, which possesses B genome most likely differentiated from S genome of *Ae. speltoides*.

The preferential amplification, transcription, and post-transcriptional processing of primary transcripts observed in tetraploid and hexaploid wheat NC hybrids strongly support the control of heteroplasmy by NC compatibility. In addition, Noyszewski *et al.* (2014) reported significant heteroplasmic polymorphisms in the mitochondrial genomes of the alloplasmic durum wheat and its cytoplasm donor of *Ae. longissima*. On the other hand, Tsunewaki *et al.* (2019) have provided concrete evidence of plasmon autonomy in the alloplasmic line of hexaploid wheat with *Ae. markgarafii* cytoplasm, by showing that neither phenotypic (male sterility) nor SSR polymorphism changes have occurred in the organellar genomes of the alloplasmic line after 63 generations of backcrosses, same as in the reconstructed maternal *Ae. markgarafii* after more than 60 generations of coexistence of this plasmon with wheat nuclear genomes. The result demonstrates the persistence of plasmon autonomy even under its prolonged coexistence with the different nuclear genomes. Under natural conditions, unless rescued by mutation and/or other mechanisms, incompatible hybrids should inevitably be eliminated due to detrimental effects such as reduced plant vigor and male sterility. It can be suggested that plasmon autonomy could persist once a compatible relationship was established or incompatibility was overcome. Heteroplasmy can generate a phenotypic variation on which natural selection may act. Hence, it represents an adaptation mechanism that helps rescue functional NC compatibility by alleviating detrimental conflicts otherwise occurring at the early stages of backcrosses. The precise mechanism and significance of the dynamic cytoplasmic genomes and their interaction with nuclear genomes needs to be further clarified.

Cytoplasmic diversity and NC interaction as potential sources for NC heterosis

Kihara (1979, 1982) proposed NC heterosis based on the observations that NC hybrids with *Ae. tauschii* cytoplasm showed earlier heading and maturity than the control lines, and NC hybrids with *Ae. geniculata* Roth cytoplasm showed delayed maturity and altered plant growth habits from spring-type to winter-type. Targets of research on interspecific and intergeneric diversity in cytoplasmic genomes and their interactions with nuclear genomes have now been extended to more comprehensive ranges of plant phenotypes, including various adaptive traits. Examples of NC heterosis can be found in hexaploid NC hybrids with the cytoplasm of *Ae. crassa* or *Ae. ventricosa*, which show agronomically beneficial phenotypes such as high grain quality, high yield, tolerance to salinity, and fungal pathogen Puccinia, and in NC hybrids with the cytoplasm of *T. timopheevii* or *S. cereale*, which are characterized by their tolerance to drought stress and high gluten quality (Truvacheeva *et al.* 2021). Soltani *et al.* (2016) showed that *Ae. mutica* cytoplasm could modify the magnitude of quantitative trait loci (QTLs), controlling several physiological traits, including dry matter weight. They detected modified methylation profiles in several polymorphic regions affected by cytoplasm types. They suggested that novel NC interaction can potentially trigger an epigenetic modification cascade of nuclear genes, eventually resulting in changes in the genetic network controlling physiological traits. NC interaction can also control environmental effects on phenotypic display. For example, hexaploid wheat hybrids cv. Norin26 with the cytoplasm of *Ae. crassa*, *Ae. juvenalis* or *Ae. vavilovii* exhibit photoperiod-sensitive cytoplasmic male sterility (PCMS) associated with pistillody under long-day conditions (Murai and Tsunewaki 1993). In contrast, NC hybrids of CS do not show pistillody due to a single dominant fertility restorer gene *Rfd1* present on the short arm of chromosome 7B (Murai *et al.* 2002). PCMS in Norin26 should provide an attractive genetic system for understanding more environmental effects on NC interaction.

Interspecific and intergeneric diversity in cytoplasms exerted on nuclear transcriptomes and metabolomes was compared among hexaploid wheat hybrids with cytoplasms of *Aegilops uniaristata*, *Ae. tauschii* or a wild barley species *Hordeum chilense* (Crosatti *et al*., 2013). They showed that the NC hybrids with *H. chilense* cytoplasm induced higher degrees of transcriptome and metabolite alterations than those in the others, reflecting their phylogenetic distances. We studied the extent of cytoplasmic and nuclear genome diversity affecting sensitivity to submergence stress using a collection of 37 NC hybrids, in which distinct cytoplasms of the *Triticum-Aegilops* complex were combined with a common nucleus of CS and 12 hexaploid wheat nuclear donors covering a wide range of genetic diversity (Takenaka *et al.* 2018). The study revealed the presence of marked phenotypic variabilities among NC hybrids and nuclear donors, demonstrating divergent cytoplasmic and nuclear effects on submergence stress tolerance. NC hybrids with *Ae. mutica* cytoplasm showed less sensitivity, whereas hybrids with cytoplasm of *Ae. umbellulata* and its related species showed greater sensitivity. Submergence stress markedly increased superoxide dismutase (SOD) activity in the susceptible hybrids, suggesting that the elevated SOD activity has resulted from high levels of accumulated reactive oxygen species. Comparative studies of submergence and the effects of seed aging on germination and seedling growth were also made using hexaploid wheat NC hybrids (Takenaka *et al.* 2019). Magnitudes of inhibition of shoot growth by submergence stress and seed aging varied greatly among NC hybrids, and their combination caused severe leaf chlorosis in most hybrids. The results indicate that cytoplasmic genomes greatly affect seed germination and seedling growth under unfavorable stress conditions and that effect of such stress is enhanced with seed aging. To examine possible NC interaction, a further study is necessary by using NC hybrids of diverse nuclear genomes combined with given cytoplasmic genomes.

Control mechanisms against unfavorable environmental stresses have been challenged by the coordination of gene expression between nuclear and cytoplasmic genomes. Nuclear genomes exert far dominating roles in controlling all cellular processes, yet intact and functional cytoplasmic genomes are needed for the normal functioning of nuclear genomes. Several studies have demonstrated significant NC or cytonuclear interactions that affect various phenotypes in *Arabidopsis thaliana*. A study using reciprocal recombinant inbred lines showed that cytoplasmic genome variation and NC interaction greatly affect natural variation in metabolomes at the intraspecific level (Joseph *et al.* 2013). NC interactions affecting adaptive traits in the fields have also been demonstrated in 56 cytolines (synonym of NC lines) produced from eight natural *A. thaliana* accessions (Roux *et al.* 2016). An important implication of these studies is that a wide range of plant phenotypes are likely under control of NC interaction, particularly through retrograde regulation in that some yet unknown molecular signals generated and excreted from cytoplasmic genomes can regulate expression of nuclear genomes. Retrograde signaling has been suggested to regulate transcriptional activities of nuclear genomes in response to organellar activities in many eukaryotic organisms (Liu and Butow 2006, Chan *et al.* 2016). However, the roles of cytoplasms and the genetic system of complex NC interactions in regulating adaptive traits yet remain largely uninvestigated, even though they have long been suggested to be important in stress tolerance, signaling, and adaptation in plants (Bock *et al.* 2014, Dobler *et al.* 2014, Mielecki *et al.* 2020). Further research towards a more precise understanding of the genetic system, including signal transduction pathways involved in NC compatibility, is needed before further manipulating and utilizing diversity in cytoplasmic genomes and NC interactions. For this, versatile methodologies such as cytoplasmic genome transformation, editing, and/or induction of recombination need to be developed and available.

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