THE COMMUNICATION OF ENDOGENOUS BIOMOLECULES (RNA, DNA, PROTEIN, HORMONE) VIA GRAFT UNION MIGHT PLAY KEY ROLES IN THE NEW TRAITS FORMATION OF GRAFT HYBRIDS

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Abstract

Plant graft hybridization is a fact of sexual hybridization in which heritable changes trigger by grafting, it can produce hereditary variation types desired by breeders and can be used as a new method for germplasm innovation. This paper aimed to discuss new phenotypic variations such as phenotypic diversity and polyploidy formation discovered in recent years, new evidence supporting genetic material exchange between the rootstock and scion, and the timeliness, direction, and genetic stability of trait formation induced by graft hybridization. The unresolved mechanisms of trait variation were also discussed, and the relationship with exchange of chloroplast DNA (cpDNA) genetic information, horizontal gene transfer events of chloroplast and mitochondria, miRNA-mediated post-transcriptional regulation, and pressure-driven trait variations from graft hybridization were performed to clarify the relevant questions. Finally, the future research trend and key questions of graft hybridization were identified. Three clear conclusions for graft hybridization are presented: the new traits of graft hybrids have stable genetic characteristics and can be controlled via selection different genetic plant by grafting, and the products of graft hybrids were safer than those of transgenic plants.

Key words: Plant graft hybridization; Graft hybrids; Phenotypic variation; Hereditary character.

Introduction

Plant grafting is a method for not only seedling propagation and preservation of germplasm resources (Wang 2011a; Wang et al., 2016a), but also cause changes in the traits of graft hybrids, which are stably inherited (Liu, 2006; Liu, 2013a; Zhou and Liu, 2015). These characteristics include phenotypic traits such as leaf color, leaf type, epidermal hair, and stem height (Li et al., 2013; Tetsumura et al., 2015; Khadivi-Khub & Anjam, 2016; Warschefsky et al., 2016), flowering and juvenile periods (Chang et al., 2010), and stress resistance(Abbas et al., 2008; Temperini et al., 2013; Calvo-Polanco et al., 2016). Moreover, these characteristics can influence the yield and quality of produce (Wu et al., 2014; Xu et al., 2014; Soares et al., 2015; Riga et al., 2016) and improve physiological metabolism processes (Zhu et al., 2010; Zhou, 2014; Huang et al., 2015), thereby contributing to a new method for germplasm creation (Zhang et al., 2002; Wang, 2011a; Wang et al., 2011b; Zhu et al., 2012; Liu et al., 2013b). These phenomena might be attributed to genetic material exchange between scions and rootstocks (Stegemann & Bock, 2009; Liu et al., 2010; Wang et al., 2011b; Li et al., 2013; Duan et al., 2014; Sun et al., 2014; Wang et al., 2016a), leading to the formation of new traits from grafting, or adaptive variation caused by the selection pressure of graft hybridization (Wang, et al., 2011b; Liu, 2013a; Huang et al., 2015). Alternatively, graft hybridization might result in genomic (Zhang, 2002; Moreno et al., 2014) and mitochondrial DNA methylation of both rootstocks and scions (Lewsey et al., 2016), leading to the silencing of functional genes (Bazzini et al., 2007). Numerous studies have shown that grafting leads to a two-way communication of chloroplast, mitochondrial horizontal gene transfer (HGT) (Stegemann et al., 2012; Hao et al., 2014; Gurdon et al., 2015), proteins, and other signal molecules across the graft union (Liu, 2013a; Duan et al., 2014; Melnyk & Meyerowitz, 2015; Wang et al., 2016b).

With the advent of new research technologies, a series of groundbreaking findings was made for determining the mechanism underlying the traditional technique, grafting. These results have promoted development of grafting for scientific direction. Thus, plant graft hybridization might become a new option for generating innovative germplasms (especially for plant breeding among internal or external species, genera and families), promoting species diversity, and ensuring food safety for the future (Albacete et al., 2015).

Changes in phenotypes, physiologies, and genetic characters by graft hybridization is a common fact: Graft hybrids have significantly altered phenotypic traits. The Compendium of Materia Medica, Darwin’s “Pangenesis,” and Michurism have recorded the phenomenon of graft hybrids (Liu, 2006; Liu et al., 2013b), which has rebuilt the interest of the scientist over the past 20 years. Fan (1999) found that graft Prunus japonica onto P. armeniaca rootstock can induce the rootstock to produce a P. japonica-like phenotypic variation. Grafted herbaceous Solanum pimpinellifolium onto the woody Lycium chinense showed that the vegetative hybrids from intergeneric graft hybridization (heterografting) were different with the same species (auto grafting). The fruit size, shape, weight and color of hybrids were all influenced by the rootstock of L. chinense, and the quality of the fruits was improved. After the scion of Gossypium hirsutum was grafted onto the Gossypium barbadense rootstock, the flowers and seeds showed mutations: the base of the flower changed from the yellow with red spots (Hao et al., 2014). The hairs on the seed coat disappeared, and they were replaced by numerous short fibers (Hao et al., 2014). The vegetative hybrids exhibited intermediate characteristics between G. hirsutum and...
and \textit{G. barbadense}. The grafting between \textit{Nicotiana glauca} and \textit{N. tabacum} produced new polyploid species (belonging to graft chimera), and the plant height and leaf length of the new hybrid species were increased (Fuentes et al., 2014). However, the phenotypic traits of the leaves and flowers as well as their size and color showed intermediate characteristics between the scion and rootstock. Thus, the phenotypic trait variation of this type tends to be of intermediate state between the scion and rootstock.

Graft hybridization can significantly alter the physiological processes of graft hybrids. For example, the net photosynthetic rate of pericinal chimeras from \textit{Brassica juncea} and \textit{B. oleracea} hybridization was 24.8% higher than that of the control cabbage group (Zhu et al., 2010). In addition, the pericinal chimera produced a new leaf shape that was stably passed to its offspring. The rate of shoot apical meristem differentiation decreased from 74.52% to 3.01% after three successive generations of self-fertilization (Li et al., 2013). A similar finding was noted in walnuts. Three years after the scion of walnut \textit{Juglans regia} cv. Shangsong 14 (‘Shangsong’) was grafted onto ‘Zhongningqi’ (bred from Juglans hindsi × \textit{J. regia}) and \textit{J. regia} cv. Ningyou (‘Ningyou’) rootstocks, the height of the ‘Zhongningqi’ vegetative hybrid reached 5.33 m, whereas that of the ‘Ningyou’ hybrid was only 4.18 m, which corresponded to an increase of 27.5% of the rootstock ‘Zhongningqi’ (Zhou, 2014). Further, the two vegetative hybrids showed significant difference in photosynthesis. The net photosynthetic rate and chlorophyll obtained by graft hybridization between \textit{S. pinnellifolium} and \textit{L. chinense} were significantly higher than auto-grafting tomatoes (Huang et al., 2015).

Graft hybridization can significantly alter the genetic characteristics of graft hybrids. In \textit{Camellia}, when single-flowered \textit{Camellia changii} Ye was grafted onto the double-flowered \textit{C. japonica} cv. Hongluzhen, the expression of B-function gene was increased, leading to an increase in the number of petals in \textit{C. changii} (Zhu et al., 2011). Thus, grafting transformed single-flower to semi-double flower ones. This approach has expanded the origin of double-flower in \textit{Camellia}. The grafting of plum \textit{Prunus cerasifera} cv. Pissardi (‘Pissardi’) onto the green-leaved \textit{Prunus salicina} cv. Yuhuang (‘Yuhuang’) produced some graft hybrids with evident traits of red leaves. However, this phenomenon was not observed in the auto-grafting of the offspring of ‘Yuhuang’ (Zhou et al., 2013), which suggested that the red leaf trait of ‘Pissardi’ (gained from grafting) was inherited stably by the graft hybrids. The graft \textit{Nicotiana glauca} with 24 chromosomes on \textit{N. tabacum} with 48 chromosomes led to the formation of a new graft chimera with 72 chromosomes (Fuentes et al., 2014). When graft \textit{N. tabacum} with mitochondrial-regulated male sterileleon the fertile \textit{N. sylvestris}, the reproductive capacity of \textit{N. tabacum} was restored at the graft union. Moreover, the horizontal transfer of mitochondrial DNA was observed, indicating that grafting accelerates the modification of mitochondrial DNA (Gurdon et al., 2015). After the susceptible tobacco scion was grafted onto the transgenic anti-cucumber mosaic virus (CMV) rootstock, the vegetative hybrids gained significant HR (highly resistant) capacity. The HR ratio increased from 66.7% of the T0 generation to over 80.0% of the T1 generation (Bai et al., 2016). The resistance acquired from graft hybridization might be attributed to heterografting that upregulated the stress-related gene expression (Cookson et al., 2014).

Thus, the rootstock and scion are known to influence each other, which can produce a series of changing of traits (phenotype). Plant graft hybrids were created from distant genetic relationship which resulted in the phenotypic, physiological, and genetic characteristics generated denoted more obvious variation. Conversely, the changes were more closely genetic relationship more smaller and less obvious. Plant graft hybridization breaks the reproductive limits of sexual hybridization and can lead to the inheritable variations at a high probability. In \textit{Vigna radiate} and \textit{Impatiens balsamina} \textit{L}, graft hybrids, a 4% probability exists that phenotypic variation will occur, and variations occur in 6.25% of the inter-simple sequence repeat (ISSR) marker amplified bands (Zhao et al., 2013). The mutation probability in the progenies of the red-leaved vegetative hybrids from ‘Pissardi’ and ‘Yuhuang’ was approximately 2.3–15.8% (Zhou et al., 2013). Nonetheless, questions such as what is the mechanism of variation in graft hybrids; is stable inheritance of modified traits possible; and can the traits be artificially regulated have long puzzled the academia?

\textbf{Exchanges in genetic material between rootstock and scion by graft hybridization through graft junction:} Plant graft hybridization can result in the transfer of cpDNA genetic material and horizontal transfer of nuclear DNA between the rootstock and scion, but nuclear gene exchange does not occur. (Stegemann et al., 2012) grafted transgenic tobacco with different resistance marker genes and reporter genes. In one group, the marker genes were mapped to the nuclear genome; however, in the other group, the marker genes were located in the chloroplast genome. After grafting, the fluorescent color reaction showed that the marker genes were frequently carried to the chloroplast genome at the graft union. The phenomenon of the exchange of these genes only occurred in this region, but not over a long-distance. (Fuentes et al., 2014) simultaneously grafted two tobacco species with different chromosome numbers. In addition to the observed changes of phenotypic traits in graft hybrids, the graft union also produced a new heterologous polyploid (graft chimera), the \textit{N. tabacca}, having a chromosome number that was the sum of the two species. However, they believed that it was HGT that caused this phenomenon, and nuclear genetic information exchange did not occur. (Tsaballa et al., 2013) grafted \textit{Capsicum annuum} cv Piperaki Long (‘Piperaki’) with elongated fruit shape on \textit{C. annuum} cv Mytlin Round (‘Mytlini’), which has a round fruit shape. The results showed that the fruit shape of the vegetative hybrids consecutively changed for two generations. However, the \textit{CaOvate} gene sequence that controls the shape of pepper did not change. These findings indicated that, even though no exchange of genetic material occurs between graft hybridization, an unknown endogenous driving force can lead to phenotypic variation in graft hybrids. Thus, what are the factors that lead to this series of events? What are their respective roles?
NEW EVIDENCES, NEW VIEWPOINTS & NEW DIRECTIONS OF PLANT GRAFT HYBRIDIZATION.

Existing research findings showed that proteins, mRNA, small RNA (sRNA), micro-RNA (miRNA), and satellite RNAs were involved in the event of grafting (Li et al., 2014; Yang et al., 2015; Chang et al., 2016; Wang et al., 2016a; Warschefsky et al., 2016). For example, in plant blossom, the FT protein (florigen) could induce plant flowering via the phloem of the graft union (Corbesier et al., 2007; Putterill & Varkonyi-Gasic, 2016), this was likely related to the high homology rate (96.93%) of florigen protein in same family (Zhu et al., 2014). However, which florigen contributed the most to the flowering of graft hybrids, from the rootstock or scion, is not yet known. If this trait is determined by one side, how is the gene of the other side silenced? What is the relationship between the two? In addition, large-scale movements of mRNA molecules were noted in graft hybrids. In the vegetative hybrids of Vitis girldiana and V. palmata, (Yang et al., 2015) found that the mRNAs of more than 3000 genes were transported up and down via the graft union. Although the movement rates of these mRNAs were between 1.0% and 65.0%, (Yang et al., 2015) believed that such large-scale movement of mRNAs would induce complex biological responses. (Liu et al., 2016) also found differences in the expression of over 3485 genes in the vegetative hybrids of Citrullus lanatus and C. moschata cv. Shintowa. Recent studies revealed that sRNAs/miRNA of 21–24 nt can directly induce genes silencing during grafting. Further, for example, in plant blossom, because flowering pathway have been relatively clear, (Wu et al., 2013a) found that miR5200, a member of the miRNA family, regulates the expression of the FT protein. The induced expression of miR5200a by short daylight leads to the methylation of the FT gene and blocks florigen synthesis, thereby delaying flowering in Brachypodium distachyon. This indicates that miRNAs regulate the expression of plant proteins after transcription. Thus, the molecular regulation of flowering is moved to the post-transcriptional stage, which ultimately affects the expression of florigen. In the methylation map constructed using epIRL (epigenetic recombinant inbred line) in Arabidopsis thaliana, three quantitative trait loci that might be associated with FT gene expression were found to be methylated (Cortijo et al., 2014). This finding indicates that grafting between two plants can directly lead to silencing of the FT gene for one side, thereby altering its flowering. (Lewsey et al., 2016) further showed that the interaction of miRNA in graft hybrids promoted the exchange of epialleles between the rootstock and scion, leading to the silencing of thousands of transposons. Thus, the expression of adjacent genes were affected. These suggested that the reliance of sRNAs on domains rearranged methylase DRM1 and DRM2 methylation pathways, as well as the independent chemically modified tetracycline CMT2 and CMT3 methylation pathways, resulting in a wide range of DNA methylations, causing inheritable trait variations (Lewsey et al., 2016). The indirect effect of miRNAs on the abundant accumulation of florigen might also influence flowering. Further, miR156 is known to play an important role in the maintenance of the juvenile stage of plants (Bhogale et al., 2013). In Arabidopsis (that leaves before flowering), the expression of squamosa promoter binding protein-like (SPL) is down-regulated by miR156, which indirectly affects the expression of FT gene and delays flowering (Kim et al., 2012). Following the identification of miR399, miR395, miR172, and miR156 were also identified as mobile miRNAs that can move freely in the phloem of heterografting (Bhogale et al., 2013). In Rosaceae (that typically flowers before leaf development), florigen synthesis and long-distance floral induction mechanism are different from those of monocotyledons, which synthesize florigen in the cotyledons and transport it to the apical shoot for floral induction (Yoo et al., 2013). This is possibly because of the existence of the fifth regulatory pathway, the high-temperature induction pathway, which is different from the four established pathways involved in flowering—the photoperiodic pathway, gibberellin pathway, autonomous pathway, and vernalization pathway. The synthesis of florigen can be induced by temperature-sensitive miRNAs, which upregulate the expression of the FT protein. We found that flowering can be induced in Armeniaca sibirica by exposure to >15°C temperature over 5–7 consecutive days after a 600–800 h cold-induced dormancy period. Therefore, we believe that after L. sibirica completes the winter dormancy period, high temperature induction is one of the key pathways for the abundant accumulation of florigen. The mechanism underlying this phenomenon might be related to the fact that high-temperature induction of miR399 can upregulate the expression of the FT gene, thereby promoting early flowering (Kim et al., 2011). Conversely, the flowering period remains unchanged under low temperature conditions. In the comparative miRNA analysis of the tomatol Lycium barbarum graft hybrid with the autografted tomatoes, 168 putative novel miRNAs were identified. In addition, 43 miRNAs were detected in the root and 163 were located in the fruit; these miRNAs showed significant expression differences. (Khaldun et al., 2016) indicated that a long-distance exchange of miRNA signals between rootstock and scion regulates the complex biological processes of graft hybrids.

Plant grafting causes the previously independent rootstock and scion to form an integrated graft hybrids. In this hybrids, macromolecules such as proteins and hormones, as well as small signaling molecules such as sRNAs and miRNAs, can be exchanged. Further, cpDNA undergoes genetic material exchange, and horizontal transfer of nuclear genes occurs at the graft union. Under this complex selection pressure, a series of stress-related chain reactions is likely to occur in graft hybrids. However, still many questions regarding the timeliness, directionality, and genetic stability of this reaction remain unsolved.

On the timeliness, directionality, and genetic stability of new traits in graft hybrids: The timely occurrence of trait variations of graft hybrids can be unstable, i.e., they might occur in a short time, or require longer to occur; occasionally, it might not occur at all. These phenomena might be related to the competence of the scions, or the degree of lignification. It might also be related to the developmental stage of plants—juvenile or adult. (Flachowsky et al., 2012) used micrografting techniques to graft the apple clone T355 clone with the CaMV355::gusA reporter gene onto the Malus domestica Borkh with hrrp-gusA reporter gene. The graft hybrids were cultivated under greenhouse condition, and hrrp-gusA silencing was not observed. In the G.
barbadense and G. hirsutum graft hybridization experiments, three HGTs were detected. However, trait variation was only found after three generations (Hao et al., 2014). In 1965, Fan et al. (1999) used the annual P. armeniacu as rootstock and mature P. japonica bud as a scion to produce a graft chimera. In 1986, a P. japonica-like phenotypic variation was first observed in the apricot rootstock. The phenotypic trait variations required 12 years to appear. Therefore, the occurrence of variations might require quantitative changes or the induction of appropriate conditions.

The directionality of trait variations by graft hybridization can also be difficult to determine, since it could be unidirectional or bidirectional. Most studies consider the mutual influence of rootstock and scion as a unidirectional effect. In Arabidopsis, the FT mRNA related to flowering was transferred from the rootstock to scion in order to influence flowering (Li et al., 2011). In J. regia experiments performed by Zhou (2014), the rootstock was found to affect the photosynthesis and growth of the scions. In Arabidopsis, the auxin-related Aux/IAA mRNA became active after it was transferred from the scion to rootstock (Li et al., 2011). Fan (1999) also showed that the scion had a unidirectional effect on the rootstock, leading to trait variations resembling the scion. However, some studies support the bidirectional effect. For example, (Banerjee et al., 2009) found that the StBEL5 mRNA was the key factor that was involved in the bidirectional regulation of the formation of chimeric potato tubers. (Fuentes et al., 2014) created polyploid tobacco by superimposing the rootstock and scion genomes onto each other.

The heterosis obtained by graft hybridization might be stably inherited. After Jatropha curcas was grafted onto the rootstock with extraneous gene pAnos, the random amplified polymorphic DNA technique was used to confirm that the genetic stability of the graft hybrid could reach 100% (Jaganath et al., 2014). (Zhang et al., 2002) grafted self-pollinated Vigna radiata onto cross-pollinated Impatiens batatas. The acquired seeds of vegetative hybrids showed significant genetic variation after successive generations. Although the authors did not describe the number of seeding required, their results indicated that these mutations were stably inherited for at least 3 generations in the offspring of self-pollinated mung beans. Wang (2011c) showed that the leaf shape variation of interspecific chimeras formed by purple cabbage and mustard tuber was stably inherited for 3 generations. When ‘Mytilini’ was grafted onto ‘Piperaki’, a new, oval-shaped fruit was produced, which could be stably inherited for at least 2 generations in this self-pollinated crop (Tsaballa et al., 2013). Arabidopsis thaliana, a typical self-pollinating plant, is known to have an outcrossing rate of 0.74% within the group and that of 0.1%–8.0% between groups (Cortijo et al., 2014). In the study on apiRIL, environmentally induced epiallele state stability, phenotypic variations caused by the epiallele were found to exist stably in A. thaliana for at least 8 generations (Cortijo et al., 2014). Similarly, when the CMV-infected tomato was grafted onto the rootstock of resistant transgenic tomato, the acquired viral resistance material was retained for at least 2 generations (Bai et al., 2016). However, the level of resistance increases with each generation. In our study, after the Vitis amurensis scion was grafted onto Schisandra sphenanthera rootstock, variations were found in the leaf size, vein shape, leaf hair, leaf color, fruit traits, flowering, and fruit development period of the vegetative hybrid (Fig. 1). These traits remain unchanged and can be stably inherited after cutting propagation.

![Fig. 1. A new graft hybrids union between Vitis amurensis (scion) and Schisandra sphenanthera (rootstock).](image-url)

(A) Vegetative hybrids from Vitisamurensis and Schisandra sphenanthera (VSc). (B) Graft union. (C) The fruits of vegetative hybrids from VSc. (D) V. amurensis has a longer internode. (E) The internode length of VSc is between those of V. amurensis and S. sphenanthera. (F) The internodes of S. sphenanthera are shorter. (G) The leaf phenotype of S. sphenanthera. (H) The leaf phenotype of V. amurensis. (I) The leaf phenotype of VSc shows that the leaf vein is change from reticulate to radial shape. (J) The fruits of S. sphenanthera. (K) The fruits of V. amurensis. (L) The characteristics such as size and pigmentation of the fruits of VSc differ according to the scion and stock. The arrow shows the difference in places between scion and stock, the scale plate of background for figs. 1D-1F, 1J-1L indicate 1 cm.
Discussions

One of the main reasons for changes in leaf traits could be cpDNA genetic material exchange at graft union:
Plant cpDNA is known to be highly conserved because the impact of external selection pressure on cpDNA is considerably small. In rice, the mutation rate of cpDNA is only one-tenth of that of the nuclear genome (Wang, 2011a). However, cpDNAs regulate some very important plant traits such as the leaf spot color and their inheritance (Hagemann, 2000), and photosynthetic efficiency (Peng et al., 2017). Thus, the creation of new cpDNA varieties can be a challenging task. (Stegemann et al., 2009) showed that graft hybridization could accelerate the probability of cpDNA varieties. (Zhou et al., 2013) found that, after grafting ‘Pissardii’ to ‘Yuhuang,’ the seedlings of the offspring of ‘Yuhuang’ exhibited an evident trait of violet leaves. We think that this phenomenon could be attributed to the imbalance in the regulation of gene expression controlling tetrapyrrole and carotenoid metabolism in the chloroplast (Wang, 2015). Alternatively, the exchange and formation of new genes or proteins could result in abnormal color expression in the graft hybrids. This implies that graft hybridization might lead to the exchange of cpDNA genetic material at the graft union, thereby affecting the variation and inheritance of leaf color. This finding might become a new method to determine the mechanism of leaf color formation and plant breeding. (Zhu et al., 2010) and Zhou, 2014) found that graft hybridization caused difference in the photosynthetic mechanism and biomass of the graft hybrid. This could be because of the regulation of the ATP synthase gene expression of the chloroplast at the graft union because ATP synthase exhibits a relatively high mutation rate (Wang, 2011a), thereby promoting adaptive mutation to also occur in its chloroplast genome in the graft chimeras.

The signal substances at the synthesis part (rootstocks or scions) determine the new traits of receptor part (scions or stocks) in graft hybrids: The scion and rootstock contribute differently to the graft hybrids (Kümpers et al., 2015; Nelson, 2004), leading to the formation of a primary and secondary relationship in the determination of graft hybrid characteristics. In plants, the synthesis of some signal substances is known to be determined by specific locations. Thus, we think that the synthetic location of these signal substances determines the phenotypic trait variation of the receptor.

We hypothesized that the scions were located in the upper part of the plant, where they could receive more light/photoperiod signals, temperature signals, and exogenous pollen than the rootstock. Thus, photosynthetic products and signal substances were continuously transported down to the rootstock. Because signal transmission occurs in an upper position, the scion became the “transmitter” of the signaling materials. Conversely, the rootstock only provided nutrients, water, and related substances to the scion. The signal transmission occurred in a lower position, and hence the rootstock cells were in a state of receiving. Thus, they became a “receptor” of signals. Moreover, in the study by (Fan et al., 1999), as well as in actual production, ensuring that scions receive sufficient nutrients, requires that all the lateral branches of rootstocks are removed. This action further weakened the abundance of signal substance production in the rootstocks. Therefore, we believe that the rootstock was more affected by the scion. This resulted in the ability of the scion to determine the trait of the rootstocks. This perspective is different from that of (Taller et al., 1998) who proposed that rootstock tends to decide the variation of scion’s phenotypic traits.

The miRNAs negatively regulate the expression of target genes after transcription at the synthesis part (Liu, 2013a) that notably affects the trait of the “receptor” miR319 can regulate the formation of Arabidopsis leaves by influencing the expression of genes Lancingel (Ori et al., 2007) and Knotted1-like homeobox (Li et al., 2012). In addition, miR161/166 negatively regulates the HD-ZIP transcription factor by forming a complex with the argonaute protein, which affects the differentiation and development of A. thaliana apical meristem cells, resulting in the changes in the morphological development of leaves (Sun et al., 2012; Zhang et al., 2013). External heat stress induced the expression of ABA in rootstocks, leading to inhibition of the expression of csa-miR159b in cucumbers. Moreover, the expression of target genes such as CgAMYB1/CsMYB29, and CSHSP70 was up-regulated, which improved heat resistance of the graft hybrids (Li et al., 2016). In addition, the difference of preferential rootstocks to nutrient elements, water content and miRNA can affect the nutrient and water requirement of the scions, which can significantly restrict the formation of scion-like phenotypic traits. Thus, the graft hybrids often show rootstock-like phenotypic variations. For example, graft ‘Mytilini’ onto ‘Piperakii’ led the fruit shape to become an intermediate between the two varieties, i.e., the round fruit of ‘Mytilini’ was significantly elongated in the longitudinal axis and reduced in the horizontal axis, thereby forming an oval shaped fruit (Tsaballa et al., 2013). Intergenic grafting of Solanaceae and Lycium in the study of (Huang et al., 2015) showed that trait variation of graft hybrid existed between tomato and wolfberry, e.g., the fruit color pigmentation changed from that of tomato to that of wolfberry. Hence, the formation of new characteristics was at an intermediate state between the two. Although the authors did not conduct studies at the molecular level, we deduced that the molecular mechanism underlying this phenomenon might be related to the miRNA produced by wolfberry (rootstock) that caused the silencing of genes that regulated the traits (scion) of tomato via DNA methylation. Further, (Khalidun et al., 2016) found that the Solyc07g06284.2, Solyc11g027650.1, and Solyc03g121000.2 genes in the wolfberry/tomato hybrid and three equivalent miRNAs with unknown functions tono50, tono95, and tono97 could be involved in the long-distance transport from the rootstocks to scions thus the traits of rootstock determined the scion in a graft hybrid. This result is the same as that of (Taller et al., 1998), but different from the wide-spread DNA methylation events caused by the transfer of sRNA from the scion to rootstock as observed in the auto grafting of Arabidopsis by (Lewsey et al., 2016). It is also very unlikely to be related to HGT, as revealed by Stegemann & Bock (2009) and (Fuentes et al., 2014). This could be because, during the intergenic grafting with large phenotypic trait differences, the resultant DNA methylation event has a stronger deterministic effect on trait variations than auto grafting or HGT (limited to the graft union).
The long-distance transport of hormones through the graft union plays an important role in the graft hybridization signal transduction (Kümpers et al., 2015; Wang et al., 2016b). Therefore, the hormone-dependent trait variation depends on whether the hormone is synthesized in the scion or rootstock. Cytokinin is synthesized in the plant roots and transported to the apical meristem through the phloem. Conversely, auxin is synthesized in the plant leaves and transported to the roots via the phloem for its physiological action. The ethylene precursors (ACC) are synthesized in the xylem of the rootstock and transferred to the scion via the graft union, which simultaneously inhibit the biomass accumulation of the *Solanum pimpinellifolium* leaves and increase the water use efficiency of chimeras (Cantero-Navarro et al., 2016), reduce the dependency for potassium, and improve the growth in soil lacking potassium (Martínez-Andújar et al., 2016). Therefore, we speculate that, for the determination of graft hybrid phenotypes, the traits maintained by cytokinin and ethylene are determined by the rootstock for the scion, and the directionality of the new trait is closer to the rootstock. Conversely, the traits maintained by auxin are influenced by the scion, and the new trait variations in the rootstock are closer to the scion in terms of orientation. This phenomenon might be related to the expression of some genes related to plant hormones, which affect the formation of new traits or trait variations in graft hybrids. Fundamentally, because of the basic needs of plants for developmental stage progression or trait maintenance, the endogenous driving factors induced by the synthetic sites need to determine the directionality of the traits of graft hybrids.

**Fig. 2.** Schematic viewpoint of the molecular mechanism of graft hybridization.
NEW EVIDENCES, NEW VIEWPOINTS & NEW DIRECTIONS OF PLANT GRAFT HYBRIDIZATION.
The traits of graft hybrids can be designed by human control: In general, the variation in phenotypic traits in graft hybrids tends to be at an intermediate state between the rootstock and scion, with a tendency toward rootstock-like traits. However, the overall trends of new traits tend to compromise the phenotypic traits of the rootstock and scion, resulting in the intermediate state. This indicated that the general direction of variations of graft hybridization traits could be achieved by artificial control.

The agricultural products from graft hybrids are safer than transgenic plants: While scions can be grafted onto transgenic rootstocks to obtain the desired traits of graft hybrid could no doubt follow the rule of natural selection (cytomembrane filtration) and compatibility. Moreover, this approach reduced the public concern regarding transgenic food safety, considering that, comparison of the desired traits obtained from graft hybridization with those obtained from transgenic techniques shows that the former clearly involves an additional step of natural selection in the later development in graft hybrids.

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Reference


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