Grafting-Induced Gene Expression Change in *Brassica rapa* Leaves is Different from Fruit Trees

Myeong-il Mun¹, Hankuil Yi¹, Ill-Sup Nou², Yoonkang Hur^{1*}

¹Department of Biological Science, College of Biological Sciences and Biotechnology, Chungnam National University, Daejeon 305,764, Korea

²Department of Horticulture, Sunchon National University, Suncheon city, Jeonnam 540-742, Korea

ABSTRACT Grafting has widely used in the agriculture of fruit-bearing crops and trees because rootstocks have a profound influence on many aspects of scion development and scion responses to the environmental changes including biotic and abiotic stress tolerance. These effects appear to result from the change of gene expression on scion, but only limited numbers of papers have been published demonstrating it. To identify altered expression of genes in Chinese cabbage, Chiifu (*Brassica rapa* ssp. *pekinensis*, inbred line) shoot was grafted on three *Brassica* rootstocks: mustard, turnip and broccoli. After head formation, Br300K microarray experiment was conducted using total RNAs from scion leaves collected in two different seasons, spring (June) and fall (October). A large number of differentially expressed genes (DEGs) were identified both in two seasonal samples, but DEGs were more notable in June sample than in October sample. However, the number of DEGs by three rootstocks were high in October with respect to up-regulation, but high in June for down-regulation. Categories of DEGs included metal ion binding, response to hormonal stimuli, response to endogenous stimuli, regulation of transcription, oxidation reduction and response to stress. Up-regulated genes in both June and October samples were similar in mustard and turnip rootstocks, but different in broccoli rootstock. Two genes were found to respond to all experimental conditions: *Brapa_ESTC049008* (hypothetical protein) as an up-regulated gene and *Brapa_ESTC016027* (*CNGC12*) as a down-regulated gene. Together with the previous reports, these results suggest that grafting-induced gene expression depends on the species involved.

Keywords Microarray, Chiifu, heterografting, *MBP2*, *CNGC12*

INTRODUCTION

Grafting has widely used in the agriculture of fruit-bearing crops because rootstocks are known to provide scion biomass and quality as well as biotic and abiotic stress tolerance (Lee 1994, 2003; Cleaswater *et al.* 2006; Kubota and McClure 2008; Lee *et al.* 2010; Gregory *et al.* 2013; Koepke and Dhingra 2013). Grafting also affects pH, fragrance, color, sugar and carotenoid contents, and texture in fruits of Cucurbits and tomatoes (Davis *et al.* 2008). As a result, most grafting studies have focused on Cucurbits, tomato, grape and fruit trees, with little attention to the vegetables.

Since grafting connects vascular tissues between rootstocks and scions, various substances can be exchanged. Among

others, minerals, amino acids, carboxylic acids, ions, phenylpropanoids and hormones could be transported from rootstocks to scions by xylem sap (Gollan *et al.* 1992; Goodger *et al.* 2005; Alvarez *et al.* 2008; Wu *et al.* 2009). In addition, various proteases are also transported through xylem sap (Biles and Abeles 1991; Satoh *et al.* 1992; Bhutz *et al.* 2004; Kehr *et al.* 2005; Alvarez *et al.* 2006). Various materials could also be transported through phloem by grafting (Le Hir *et al.* 2008): carbohydrate, amino acids, hormones and mRNAs (Ruiz-Medrano *et al.* 1999; Wu *et al.* 2002; Banerjee *et al.* 2006; Lough and Lucas 2006; Kehr and buhtz 2008), small RNA (Yoo *et al.* 2004; Pant *et al.* 2008), and proteins (Corbesier *et al.* 2007; Lin *et al.* 2007; Weller *et al.* 2009).

It was suggested that many developmental phenomena

Received March 20, 2015; Revised March 23, 2015; Accepted March 23, 2015; Published March 31, 2015

*Corresponding author Yoonkang Hur, ykhur@cnu.ac.kr, Tel: +82-42-821-6279, Fax: +82-42-822-9690

induced by grafting are caused by long-distance transmissible substances *via* phloem and xylem: flowering (Zeevaart 1976; Lang *et al.* 1977), tuber formation (Jackson 1999), nodule formation (Oka-Kira and Kawaguchi 2006), leaf development (Kim *et al.* 2001), lateral shoot formation (Beveridge 2006) and defense (Palauqui *et al.* 1997). Recent articles demonstrate that flowering is associated with long-distance transmissible substances, such as *CONSTANS* mRNA (Ayre and Turgeon 2004), *FT* mRNA (Huang *et al.* 2005), *FT* protein (Corbesier *et al.* 2007; Lin *et al.* 2007), and *GIGANTEA* and *ELF4* proteins (Weller *et al.* 2009). *FT* and *siRNAs* in phloem, which lead epigenetic regulation, can be applied in crop breeding (McGarry and Kragler 2013).

A few reports have reported scion gene expression change upon grafting (Jensen *et al.* 2003, 2010; Prassinis *et al.* 2009; Cookson and Ollat 2013). It was showing that several categories of genes are differentially expressed in genetically identical apple scions, when grafted on rootstocks with different genotypes. Differentially expressed genes (DEGs) include those responding stimulus and abiotic and biotic stress, in addition to genes with unknown biological processes and other biological processes (Jensen *et al.* 2010). Prassinis *et al.* (2009) used cDNA amplified fragment length polymorphism to compare the effect of a dwarfing and a semi-vigorous rootstocks on gene expression in the scion and observed the differential regulation of various transcription factors and genes involved in signaling processes. In addition, Cookson and Ollat (2013) demonstrated that heterografts induce more stress-responsive genes than autografts in grapevines.

Genus *Brassica* belongs to the mustard family (Brassicaceae) showing various morphology and includes *B. rapa*, *B. nigra*, *B. oleracea*, *B. juncea*, *B. napus* and *B. carinata*, which are important edible crops across the world. Brassica plants include many important vegetable crops contain very potent phytochemicals: cabbage, kale, Brussels sprouts, cauliflower, broccoli, and kohlrabi, with glucosinolates, and their breakdown products (Mithen *et al.* 2000; Park *et al.* 2012). Chinese cabbage (*B. rapa* ssp. *pekinensis*) is one of the most important leafy vegetables widely used in East Asian countries. To examine the change in gene expression by grafting, we have used Chiifu inbred line (*B. rapa* ssp.

pekinensis) as scion and various rootstocks, such as Chiifu (grafting control), turnip (*B. rapa* ssp. *rapa*), mustard (*B. juncea*) and broccoli (*B. oleracea*). When comparing homo- and heterografting results, we found a larger number of genes in the leaves of scion (Chiifu leaves) are differentially expressed by hetero-grafting.

MATERIALS AND METHODS

Plant materials

Chiifu inbred line (*B. rapa* ssp. *pekinensis*) used as scion was obtained from Korea *Brassica rapa* Genome Resource Bank (KBGRB) and various rootstocks' seeds, such as turnip (*B. rapa* ssp. *rapa*), mustard (*B. juncea*) and broccoli (*B. oleracea* Italica), were purchased from local market in Korea. Seeds were sown in 50-hole pot and grown in a greenhouse for 2 weeks until the first foliage leaf was emerged. Grafting was performed with Y-type and resulting plants were put into a growth room with controlled moisture over 90%. After acclimation, grafted-seedlings were transplanted to soil and grown for 2 months until heads were formed. Leaf tissues from outer and inner leaves of scion were sampled from 5 independent plants in June 4 for spring and October 29 for fall growing plants, and frozen immediately in liquid nitrogen.

RNA extraction and microarray

Total RNAs were extracted after combining outer and inner leaves of scion using TriZol reagent (Invitrogen, USA) and further purified using an RNeasy MinEluteTM Cleanup Kit (Qiagen, Germany). For microarray experiment, 300k microarray chip (Version 2.0) for *B. rapa*, which was designed from 47,548 unigenes and manufactured by NimbleGen, Inc. (<http://www.nimblegen.com/>) were used (Dong *et al.* 2013). To assess the reproducibility of the microarray analysis, we repeated the experiment two times using independently prepared total RNA. The data were then normalized and processed with cubic spline normalization using quantile to adjust signal variations between chips and robust multichip analysis using a median polish algorithm implemented in NimbleScan (Workman *et al.* 2002; Irizarry *et al.* 2003). RNA preparation, GeneChip

hybridization, and data analyses were conducted as previously described (Dong *et al.* 2013).

Gene chip data analysis

Genes with adjusted p-value or false discovery rate below 0.05 were collected and further selected for those with expression difference greater than or less than at least one treatment. Multivariate statistical tests such as principal component analysis, and multidimensional scaling were performed with Acuity 3.1 (Molecular Devices, U.S.A.). Clustering analysis was carried out with MultiExperiment Viewer version 4.9 (MeV4.9, <http://www.tm4.org/mev.html>). To gain the insight on putative biological functions and biochemical pathways of DEGs, we carried out enrichment analyses by searching the Gene Ontology (GO) (Ashburner *et al.* 2000) and agriGO (Du *et al.* 2010).

RESULTSS AND DISCUSSION

Grafted Chinese cabbage

Rootstocks widely used in agriculture can have a profound influence on many aspects of scion development and scion responses to the environment (Webster 2004;

Lee *et al.* 2010; Gregory *et al.* 2013). These influences might be related to the change in gene expression by transmissible substances through phloem (Sonoda and Nishiguchi 2000; Huang *et al.* 2005; Kehr and Buhtz 2008; Notaguchi *et al.* 2008; McGarry and Kragler 2013) and xylem (Ko *et al.* 2014). Therefore, gene expression in scion can be influenced by both root and shoot parts of rootstocks. To examine the gene expression upon grafting, we used Chiifu inbred line as scion and three *Brassica* species as rootstocks. Grafted-Chinese cabbages were grown in two seasons, spring (April 1 to June 4) and fall (Aug. 10 to Oct. 29). Temperature ranges during 7 days before sampling were 13.9°C (low) - 24.7°C (high) for spring and 8.6°C (low) - 18.0°C (high) for fall. To eliminate temperature and rootstock biases, fold change in gene expression was calculated based on homografting (or autografting) values (Chiifu scion vs. Chiifu rootstock) (Fig. 1 and Table S1). As shown in Figure 1, shoot for each rootstock remained so that gene expression in Chiifu scion can be affected by mobile substances from both xylem and phloem of rootstocks. Rootstock without the shoot parts showed much less effect on scion gene expression change (data not shown).

Fig. 1. Morphology of grafted-Chinese cabbages: overall appearance (upper panels) and longitudinal section (bottom). Arrows indicate sampled tissues (outer and inner leaves) for microarray. Grafting control was Chiifu (scion)/Chiifu (rootstock). Note that rootstock and scion of the grafting control was separated before photography.

Microarray data analysis to identify DEGs

Contrast to horticultural point of view on grafting, which aims to increase the biomass and stress tolerance of scion, our experiment was conducted to examine the changes at the gene expression level, by using different rootstocks (Table S1). Hetero-grafted Chiifu scion with three different rootstocks resulted in the differential expression of a large number of genes in the scion leaves, when compared to the auto-grafted control Chiifu/Chiifu (Fig. 2). The number of DEGs was dependent on season when Chinese cabbages were grown: more genes were up- and down-regulated in the June sample compared to the October one. This might be due to high light intensity and temperature in June. Regarding to rootstocks, the differential expression of most genes was similar in mustard and turnip, but rather different in broccoli. The number of DEGs shared by all three

rootstocks used was higher in October (74 vs. 35) with respect to up-regulation, but higher in June for down-regulation (88 vs. 25) (Fig. 2). When these genes were further analyzed by functional category, most genes belonged to the categories of metal ion binding, response to hormonal stimuli, response to endogenous stimuli, regulation of transcription, oxidation reduction and response to stress (Table 1). Interestingly, genes involved in metal ion binding and response to hormonal stimuli occupied the first and second places.

Alteration of gene expression by grafting has been examined in apple trees (Prassinis *et al.* 2009; Jensen *et al.* 2010) and grape vines (Cookson and Ollat 2013). In both plants, autografting did not induce shoot gene expression, but heterografting had great effect on gene expression. However, functional category or Gene Ontology (GO)

Fig. 2. Venn diagram showing differentially expressed genes (DEGs) by three rootstocks compared to homografting. A and B represent up-regulated genes in June and October samples, respectively. C and D represent down-regulated genes in June and October samples, respectively.

terms were different between two species. In apple trees, GO terms enriched in the differentially expressed genes were response to stimulus, response to abiotic and biotic stress, among others. (Jensen *et al.* 2010). In addition, the authors identified 116 genes, whose expression levels were correlated with the plant size. Using a dwarfing and a semi-vigorous rootstocks, Prassinis *et al.* (2009) identified differential regulation for many transcription factors and genes involved in signaling processes using scion shoot apex. In grape grafting, categories of genes showing increased expression at shoot apex were genes related to DNA, chromatin structure, histones, flavonoids, and leucine rich repeat containing receptor kinases (Cookson and Ollat 2013). These results suggest that grafting-induced gene expression change is species-specific, except developmentally determined genes like those involved in FT-induced

flowering.

Identification of genes showing rootstock-dependent responses

To identify DEGs whose expression is regulated in rootstock-dependent manners regardless of growth conditions, commonly up- or down-regulated genes in both June and October samples were analyzed (Fig. 3). Similar numbers of genes were differentially regulated when mustard and turnip were used as rootstocks, but broccoli-grafted scions showed different pattern compared to the other two species. Regarding up-regulated genes, samples obtained using mustard and turnip rootstocks shared many genes, quite different from that obtained using broccoli rootstock (Table 2). The most notable genes were myrosinase-binding protein 2 (*MBP2*) and unknown protein genes.

Table 1. Functional categorization of DEGs by different rootstocks.

Functional category	Up-regulated		Down-regulated	
	June	Oct.	June	Oct.
Metal ion binding	9	23	24	8
Response to hormone stimulus	7	15	2	4
Response to endogenous stimulus	3	10	2	3
Regulation of transcription	9	6	8	1
Oxidation reduction	4	4	7	6
Response to stress	6	2	3	4
Transferase	4	2	4	10
Membrane	2	13	1	6
Response to inorganic substance	3	1	4	4
Chloroplast	0	15	2	6
Hydrolase	0	3	6	1
Monooxygenase	1	3	3	0
Signal peptide	0	6	7	3
Glycoprotein	0	3	6	2
Heat shock protein binding	3	0	1	0
Response to inorganic substance	3	1	4	4
Response to cadmium ion	2	1	0	0
Lipid transport	0	4	1	1
Lipid degradation	0	2	1	1
Response to nutrient level	1	1	1	0
Cellular response to stress	1	1	2	4
Reproductive developmental process	1	6	3	0

Table 2. Top 5 genes that were commonly regulated in both June and October samples.

Expression	Rootstock	<i>Br</i> _SEQ_ID	<i>At</i> Locus	Description	PI value		Fold change	
					Chiifu		Rootstock	
					June	Oct	June	Oct
Up-regulation	Mustard	<i>Brapa_ESTC016277</i>	AT1G52030	MBP2 (MYROSINASE-BINDING PROTEIN 2)	470	413	5,099	2,022
		<i>Brapa_ESTC007567</i>	AT1G52030	MBP2 (MYROSINASE-BINDING PROTEIN 2)	479	345	4,090	1,893
		<i>Brapa_ESTC010078</i>	AT4G27360	Dynein light chain, putative	11	19	56	138
		<i>Brapa_ESTC013929</i>	AT3G01500	CA1 (CARBONIC ANHYDRASE 1)	1460	319	4450	2079
		<i>Brapa_ESTC000600</i>	AT1G16850	Unknown protein	376	1,063	3,532	4,888
	Turnip	<i>Brapa_ESTC007567</i>	AT1G52030	MBP2 (MYROSINASE-BINDING PROTEIN 2)	479	345	8,002	2,570
		<i>Brapa_ESTC016277</i>	AT1G52030	MBP2 (MYROSINASE-BINDING PROTEIN 2)	470	413	8,909	2,364
		<i>Brapa_ESTC049008</i>	AT1G49500	Unknown protein	15	28	449	154
		<i>Brapa_ESTC026374</i>	AT5G59310	LTP4 (LIPID TRANSFER PROTEIN 4)	3,006	282	11,584	3,312
		<i>Brapa_ESTC022106</i>	AT3G01500	CA1 (CARBONIC ANHYDRASE 1)	5,941	532	20,763	5,420
	Broccoli	<i>Brapa_ESTC008104</i>	AT3G49620	DIN11 (DARK INDUCIBLE 11); oxidoreductase	605	106	4,580	645
		<i>Brapa_ESTC049008</i>	AT1G49500	Unknown protein	15	28	106	162
		<i>Brapa_ESTC026957</i>	AT3G49620	DIN11 (DARK INDUCIBLE 11); oxidoreductase	1,151	347	4,544	1,435
		<i>Brapa_ESTC047756</i>	AT3G51590	LTP12 (LIPID TRANSFER PROTEIN 12)	322	209	831	632
		<i>Brapa_ESTC028727</i>	AT3G15400	ATA20 (Arabidopsis thaliana anther 20)	150	139	327	586
Down-regulation	Mustard	<i>Brapa_ESTC016027</i>	AT2G46450	ATCNGC12 (cyclic nucleotide gated channel 12)	707	70	101	11
		<i>Brapa_ESTC022776</i>	AT1G06160	Ethylene-responsive factor, putative	4,299	466	962	71
		<i>Brapa_ESTC044452</i>	AT4G23990	ATCSLG3 (Cellulose synthase-like G3)	1,958	1,605	477	279
		<i>Brapa_ESTC022540</i>	AT5G20820	Auxin-responsive protein-related	400	474	40	232
		<i>Brapa_ESTC014184</i>	AT5G49360	BXL1 (BETA-XYLOSIDASE 1)	847	141	314	34
	Turnip	<i>Brapa_ESTC026136</i>	AT3G01420	ALPHA-DOX1 (ALPHA-DIOXYGENASE 1)	3,059	2,016	287	204
		<i>Brapa_ESTC016027</i>	AT2G46450	ATCNGC12 (cyclic nucleotide gated channel 12)	707	70	15	15
		<i>Brapa_ESTC006382</i>	AT5G45340	CYP707A3 (cytochrome P450, family 707, subfamily A, polypeptide 3)	4,649	906	962	220
		<i>Brapa_ESTC004877</i>	no_hits_found	-----	315	161	100	40
		<i>Brapa_ESTC012086</i>	AT4G16260	Glycosyl hydrolase family 17 protein	7,703	764	523	272
	Broccoli	<i>Brapa_ESTC014184</i>	AT5G49360	BXL1 (BETA-XYLOSIDASE 1); hydrolase, hydrolyzing O-glycosyl compounds	847	141	34	18
		<i>Brapa_ESTC002586</i>	AT4G35770	DIN1 SEN1 (DARK INDUCIBLE 1)	6,771	562	22	135
		<i>Brapa_ESTC016027</i>	AT2G46450	CNGC12, ATCNGC12 ATCNGC12 (cyclic nucleotide gated channel 12)	707	70	7	12
		<i>Brapa_ESTC021555</i>	AT4G15530	PPDK (PYRUVATE ORTHOPHOSPHATE DIKINASE)	4,100	604	310	100
		<i>Brapa_ESTC007363</i>	AT4G37010	Calcium ion binding	1,109	145	77	26

MBP2 has been known to form a complex with myrosinase, which degrades secondary metabolites, glucosinolates, upon wounding to provide a defense mechanism against pests. Binding of MBP2 prevents degradation of the complex, but its exact function is still unclear (Erisson *et al.* 2002). It is also not known why the expression of MBP2 is dramatically increased upon grafting in Chinese cabbage. In scions grafted to mustard and turnip rootstock, carbonic anhydrase (*CA*) gene, which regulates CO₂-controlled stomatal movement in guard cells, was also up-regulated (Table S1 and Table 1). Interestingly, up-regulated genes identified with broccoli rootstock included floral bud specific genes, such as ASK-beta (*ASK2*) and *ANAC025* (Dong *et al.* 2013). In addition, various *CYP* genes were also up-regulated when broccoli rootstock were used. Another interesting finding was anthocyanin biosynthesis-related genes were induced as a result of grafting with the rootstock of mustard (flavonol synthase, *FLS* and chalcone

synthase, *CHS*) and turnip (*FLS*), but not with that of broccoli. Down-regulated genes were variable depending on the species used for rootstocks, except cyclic nucleotide gated channel 12 (*CNGC12*) which was down-regulated in all three combinations.

Only two genes were commonly regulated in three rootstocks: *Brapa_ESTC049008* (hypothetical protein) for up-regulation and *Brapa_ESTC016027* (*CNGC12*) for down-regulation (Table 3, Fig. 3). *Brapa_ESTC049008* consists of 76 bp of 5'-UTR, 192 bp coding sequences (CDS) and 291 bp long 3'-UTR. Its CDS encodes 7 kDa hypothetical proteins with *pI* 8.0. *Brapa_ESTC049008* is 75% identical to *Arabidopsis* protein (AT1G49500) at the amino acid sequence level. *Brapa_ESTC016027* encodes *Brassica* homolog of cyclic nucleotide gated channel 12 (*CNGC12*, which is a positive regulator of resistance against avirulent fungal pathogen (Yoshioka *et al.* 2006; Urquhart *et al.* 2011). It is intriguing why grafting

Fig. 3. Venn diagrams showing differentially expressed genes (DEGs) in both June and October experiments by three different rootstocks, compared to homografting. A and B represent up-regulated and down-regulated genes, respectively.

Table 3. Genes that are differentially expressed in all experimental samples as well as both June and October samples.

<i>Br</i> _SEQ_ID	<i>At</i> Locus	Description	Fold change					
			Mustard/Chiifu		Turnip/Chiifu		Broccoli/Chiifu	
			June	Oct.	June	Oct.	June	Oct.
<i>Brapa_ESTC049008</i>	AT1G49500	Hypothetical protein	34.5	3.0	29.6	5.4	7.0	5.7
<i>Brapa_ESTC016027</i>	AT2G46450	CNGC12	-7.0	-6.1	-46.8	-4.5	-106.0	-5.9

down-regulates *CNGC12* gene, which leads to the increase in calcium sensitivity.

Recent works have focused on the mechanisms of tissue reunion during grafting, such as exchange of genetic materials in various plants (Stegemann and Bock 2009; Asahina and Satoh 2015). Although only limited numbers of papers have been reported for scion gene expression change by grafting, it was revealed that heterografts induce more stress-responsive genes than autografts (Cookson *et al.* 2014). Our results are different from previous reports since rootstocks and scions from two related vegetable species were used. To learn more about the influence of grafting on scion gene expression, gene expression needs to be examined at various growth and developmental stages.

Conclusions

Grafting Chiifu inbred line with rootstocks originating from various *Brassica* spp. had a profound effect on scion gene expression changes, when tested in two different seasons of a year. In contrast to the grafting for fruit production using vigorous rootstocks, our grafting experiment was to identify gene expression changes induced by substances from both rootstock and rootstock-bearing shoot (i.e., xylem and phloem). DEGs were more notable in June sample than in October sample. However, the number of DEGs identified by the use of three different rootstocks was higher in the samples collected in October for up-regulation, while the number was higher in June sample for down-regulation. DEGs identified includes the genes categorized for metal ion binding, response to hormonal stimuli, response to endogenous stimuli, regulation of transcription, oxidation reduction and response to stress. Up-regulated genes in both June and October samples were found similar between scions on mustard and turnip rootstocks, but somewhat different in that on broccoli rootstock. In contrast, no patterns were observed for down-regulated genes. Two genes were identified in all three experimental set-ups: *Brapa_ESTC049008* (hypothetical protein) as an up-regulated gene and *Brapa_ESTC016027* (*CNGC12*) as a down-regulated genes. Functional analysis of several genes identified in this study, using transgenic and biochemical approaches, will be necessary to further

understand molecular changes induced by grafting and improve the productivity.

Supplementary Table 1. Result of Br300K microarray. Microarray was performed with scion tissues on different rootstocks. PI values represent prove intensity of hybridization. Yellow and blue shades indicate over two-fold change in PI values.

ACKNOWLEDGEMENTS

This work was supported by Research Fund of Chungnam National University (CNU), Daejeon, Korea, to Yoonkang Hur (2014-0905-01).

REFERENCES

- Alvarez S, Goodger JQD, Marsh EL, Chen S, Aslrvatham VS, *et al.* 2006. Characterization of maize xylem sap proteome. *J. Proteome Res.* 5: 963-972.
- Alvarez S, Marsh EL, Schroeder SG, Schanchtman DP. 2008. Metabolomic and proteomic changes in the xylem sap of maize under drought. *Plant Cell Environ.* 31: 425-340.
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, *et al.* 2000. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* 25: 25-29.
- Asahina M, Satoh S. 2015. Molecular and physiological mechanisms regulating tissue reunion in incised plant tissues. *J. Plant Res.* DOI 10.1007/s10265-015-0705-z.
- Ayre BG, Turgeon R. 2004. Graft transmission of a floral stimulant derived from CONSTANS. *Plant Physiol.* 13: 2271-2278.
- Banerjee AK, Chatterjee M, Yu Y, Suh SG, Miller WA, *et al.* 2006. Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. *Plant Cell* 18: 3443-3457.
- Beveridge C. 2006. Axillary bud outgrowth: sending a message. *Curr. Opin. Plant Biol.* 9: 35-40.
- Bhutz A, Kolasa A, Arlt K, Walz C, Kehr J. 2004. Xylem sap protein composition is conserved among different plant species. *Planta* 219: 610-618.
- Biles CL, Abeles FB. 1991. Xylem sap proteins. *Plant Physiol.* 96: 597-601.

- Clearwater MJ, Seleznyova A, Thorp TG, Blattmann P, Barnett AM, *et al.* 2006. Vigor-controlling rootstocks affect early shoot growth and leaf area development of kiwifruit. *Tree Physiol.* 26: 505-515.
- Cookson SJ, Ollat N. 2013. Grafting with rootstocks induces extensive transcriptional re-programming in the shoot apical meristem of grapevine. *BMC Plant Biol.* 13: 147.
- Cookson SJ, Moreno MJC, Mendome LZN, Delrot S, Magnin N, *et al.* 2014. Heterografting with nonself rootstocks induces genes involved in stress responses at the graft interface when compared with autografted controls. *J. Exp. Bot.* 65: 2473-2481.
- Corbesier L, Vincent C, Jang S, F. Fornara, Q. Fan, I. *et al.* 2007. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316: 1030-1033.
- Davis AR, Perkins-Veazie P, Hassel R, Levi A, King SR, *et al.* 2008. Grafting effects on vegetable quality. *HortScience* 43: 1670-1672.
- Dong X, Feng H, Xu M, Lee J, Kim YK, *et al.* 2013. Comprehensive analysis of genic male sterility -related genes in *Brassica rapa* using a newly developed Br300K oligomeric chip. *PLoS One* 8: e72178.
- Du Z, Zhou X, Zhang Z, Su Z. 2010. agriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res.* 38: W64-70.
- Eriksson S, Andréasson E, Ekbom B, Granér G, Pontoppidan B, *et al.* 2002. Complex formation of myrosinase isoenzymes in oilseed rape seeds are dependent on the presence of myrosinase-binding proteins. *Plant Physiol.* 129: 1592-1599.
- Gollan T, Schurr U, Schultze ED. 1992. Stomatal response to drying soil in relation to changes in xylem sap composition of *Helianthus annuus* L. The concentrations of cations, anions, amino acids in, and pH of, the xylem sap. *Plant Cell Environ.* 15: 453-459.
- Goodger JQD, Sharp RE, Marsh EL, Schachtman DP. 2005. Relationships between xylem sap constituents and leaf conductance of well-watered and water-stressed maize across three xylem sampling techniques. *J. Exp. Bot.* 56: 2389-2400.
- Gregory PJ, Atkinson CJ, Bengough AG, Else MA, Fernández-Fernández F, *et al.* 2013. Contributions of roots and rootstocks to sustainable, intensified crop production. *J. Exp. Bot.* 64: 1209-1222.
- Huang T, Böhlenius H, Eriksson S, Parcy F, Nilsson O. 2005. The mRNA of *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. *Science* 309: 1694-1696.
- Irizarry RA, Hobbs R, Collin R, Beazer-Barclay YD, Antonellis KJ, *et al.* 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4: 249-264.
- Jackson S. 1999. Multiple signaling pathways control tuber induction in potato. *Plant Physiol.* 119: 1-8.
- Jensen PJ, Rytter J, Detwiler EA, Travis JW, McNellis TW. 2003. Rootstock effects on gene expression patterns in apple tree scions. *Plant Mol. Biol.* 493: 493-511.
- Jensen PJ, Makalowska I, Altman N, Fazio G, Praul C, *et al.* 2010. Rootstock-regulated gene expression patterns in apple tree scions. *Tree Genet. Genomes* 6: 57-72.
- Jensen PJ, Halbrecht N, Fazio G, Makalowska I, Altman N, *et al.* 2012. Rootstock-regulated gene expression patterns associated with fire blight resistance in apple. *BMC Genomics.* 13: 9.
- Kehr J, Buhtz A. 2008. Long distance transport and movement of RNA through the phloem. *J. Exp. Bot.* 59: 85-92.
- Kehr J, Buhtz A, Giavalisco P. 2005. Analysis of xylem sap proteins from *Brassica napus*. *BMC Plant Biol.* 5: 1-13.
- Kim M, Canio W, Kessler S, Sinha N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* 293: 287-289.
- Ko D, Kang J, Kiba T, Park J, Kojima M, *et al.* 2014. *Arabidopsis* ABCG14 is essential for the root-to-shoot translocation of cytokinin. *Proc. Natl. Acad. Sci.* 111: 7150-7155.
- Koepeke T, Dhirga A. 2013. Rootstock scion somatogenetic interactions in perennial composite plants. *Plant Cell Rep.* 32: 1321-1337.
- Kubota C, McClure MA. 2008. Vegetable grafting: history, use, and current technology status in North America. *HortScience* 43: 1664-1669.
- Lang A, Chailakhyan MK, Frolova IA. 1977. Promotion and inhibition of flower formation in a dayneutral plant in grafts with a short-day plant and a long-day plant. *Proc. Natl. Acad. Sci.* 74: 2412-2416.
- Le Hir R, Beneteau J, Bellini C, Vilaine F, Dinant S. 2008. Gene expression profiling: keys for investigating phloem functions. *Trends Plant Sci.* 13: 273-280.
- Lee JM. 1994. Cultivation of grafted vegetables I. Current

- status, grafting methods, and benefits. *HortScience* 29: 235-239.
- Lee JM. 2003. Advances in vegetable grafting. *Chron. Hort.* 43: 13-19.
- Lee JM, Kubota C, Tsao SJ, Bie Z, Echevarria PH, *et al.* 2010. Current status of vegetable grafting: diffusion, grafting techniques, automation. *Sci. Hortic.* 127: 93-105.
- Lin MK, Belanger H, Lee YJ, Varkonyi-Gasic E, Taoka KI, *et al.* 2007. FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the Cucurbits. *Plant Cell* 19: 1488-1506.
- Lough TJ, Lucas WJ. 2006. Integrative plant biology: role of phloem long-distance macromolecular trafficking. *Annu. Rev. Plant Biol.* 57: 203-232.
- McGarry RC, Kragler F. 2013. Phloem-mobile signals affecting flowers: applications for crop breeding. *Trends Plant Sci.* 18: 198-206.
- Mithen RF, Dekker M, Verkerk R, Rabot S, Johnson IT. 2000. The nutritional significance, biosynthesis, and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric.* 80: 967-984.
- Notaguch M, Abe M, Kimura T, Daimon Y, Kobayashi T, *et al.* 2008. Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering. *Plant Cell Physiol.* 49: 1645-1658.
- Oka-Kira E, Kawaguchi M. 2006. Long-distance signaling to control root nodule number. *Curr. Opin. Plant Biol.* 9: 496-502.
- Palauqui JC, Elmayan T, Pollien JM, Vaucheret H. 1997. Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J.* 16: 4738-4745.
- Pant BD, Buhtz A, Kehr J, Scheible WR. 2008. Micro-RNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J.* 53: 731-738.
- Park WT, Kim JK, Park S, Lee SW, Li X, *et al.* 2012. Metabolic profiling of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in Kohlrabi (*Brassica oleracea* var. *gongylodes*). *J. Agri. Food Chem.* 60: 8111-8116.
- Prassinis C, Ko JH, Lang G, Iezzoni AF, Han KH. 2009. Rootstock-induced dwarfing in cherries is caused by differential cessation of terminal meristem growth and is triggered by rootstock-specific gene regulation. *Tree Physiol.* 29: 927-936.
- Ruiz-Medrano R, Xoconostle-Cázares B, Lucas WJ. 1999. Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants. *Development* 126: 4405-4419.
- Satoh S, Lizuka C, Kikuchi A, Nakamura N, Fujii T. 1992. Proteins and carbohydrates in xylem sap from squash root. *Plant Cell Physiol.* 33: 841-847.
- Sonoda S, Nishiguchi M. 2000. Graft transmission of post-transcriptional gene silencing : target specificity for RNA degradation is transmissible between silenced and non-silenced plants, but not between silenced plants. *Plant J.* 21: 1-8.
- Stegemann S, Bock R. 2009. Exchange of genetic material between cells in plant tissue grafts. *Science* 324: 649-651.
- Urquhart W, Chin K, Ung H, Moeder W, Yoshioka K. 2011. The cyclic nucleotide-gated channels AtCANG11 and 12 are involved in multipep Ca^{2+} -dependent physiological responses and act in a synergistic manner. *J. Exp. Bot.* 62: 3671-3682.
- Webster AD. 2004. Vigour mechanisms in dwarfing rootstocks for temperate fruit trees. *ISHS Acta Hortic.* 658: 29-41.
- Weller JL, Hecht V, Liew LC, Sussmilch FC, Wenden B, *et al.* 2009. Update on the genetic control of flowering in garden pea. *J. Exp. Bot.* 60: 2493-2499.
- Workman C, Jensen LJ, Jarmer H, Berka R, Gautier L, *et al.* 2002. A new non-linear normalization method for reducing variability in DNA microarray experiments, *Genome Biol.* 3 (9): research0048.
- Wu JL, Wei ZG, Zhao HY, Li HX, Hu F. 2009. The role of amino acids in the long-distance transport of La and Y in the xylem sap of tomato. *Biol. Trace. Elem. Res.* 129: 239-250.
- Wu X, Weigel D, Wiqqe PA. 2002. Signaling in plants by intercellular RNA and protein movement. *Genes Dev.* 16: 151-158.
- Yoo BC, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S, *et al.* 2004. A systemic small RNA signaling system in plants. *Plant Cell* 16: 1979-2000.
- Yoshioka K, Moeder W, Kang HG, Kachroo P, Masmoudi K, *et al.* 2006. The chimeric *Arabidopsis* CYCLIC NUCLEOTIDE-GATED ION CHANNEL11/12 activates multiple pathogen resistance responses. *Plant Cell* 18: 747-763.
- Zeevaart JAD. 1976. Physiology of flower formation. *Annu. Rev. Plant Physiol.* 27: 321-348.