

Comprehensive analysis of the differentially expressed receptor-like kinase genes in responding to pollination in *A. thaliana* and *B. napus*

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Abstract

Background Successful sexual reproduction in flowering plant requires extensive communications between male and female organs and tissues. Although RLKs have been proved to play critical roles in these communications, so far the identified RLKs are still limited. Here we performed a comprehensive analysis on differentially expressed RLKs in responding to pollination in *Arabidopsis thaliana* and *Brassica napus* to contribute to further analysis on RLKs' function in male-female communication. Result In this study, a total of 2,583 *B. napus* AtRLK orthologs were obtained. 89 AtRLKs showed obvious expression level changes after pollination in *A. thaliana* or in *B. napus*. Although 30 differentially expressed AtRLKs were opened anthers and anthers of mature flower before opening preferentially expressed or hydrated pollen-enriched, up to 79 AtRLKs corresponding to 129 *B. napus* orthologs showed obvious expression level changes at different time points after pollination. Among 89 differentially expressed AtRLKs after pollination, only 7 AtRLKs were shared by differently expressed genes during in vitro pollen tube growth, 3 of 7 AtRLKs' expression level change tendency after pollination and during PTG were different. Conclusions Amount to 89 AtRLKs were differentially expressed in responding to pollination in *A. thaliana* and *B. napus*, and their expression level changes should be mainly induced by pollen-stigma interaction, several of them had been proved to function in male-female communication in the former reports.

Background

The stigma of flowering plants is the receptive portion of the pistil for pollination [1, 2]. Brassicaceae species have a typical dry stigma, which lacks of free-flowing secretions on its surface [3-5]. This dry type stigma has evolved sophisticated mechanisms to discriminate between compatible and incompatible pollen for successful fertilization. Once pollen lands on the dry stigma, the incompatible pollen cannot get water from the stigma, so its hydration and germination is inhibited, only compatible pollen can induce the dry stigma to release water and other factors, and can fully hydrate and germinate [5-9]. After compatible pollen germination, it must experience the following sequential processes, including pollen tube formation, penetration the intercellular space of the stigma, growth down through the transmitting tissue of the style, entry into the ovule and discharge of the sperm cells to execute double fertilization [10, 11]. Double fertilization is a unique characteristic of angiosperms, which requires a series of complicated communications between male and female reproductive organs and tissues [12-17].

Plant receptor-like kinases (RLKs) belong to a large monophyletic gene family [18, 19]. Findings in the last two decades have revealed that RLKs play critical roles in communication between male gametophyte and the female tissues during the rejection of incompatible pollen and the acceptance of compatible pollen by Brassicaceae stigmas [17, 20-23]. During rejecting self-incompatible pollen, *S*-locus receptor kinase (SRK) is the sole female determinant of self-incompatibility (SI) in Brassicaceae species [24]. SRK interacts with the pollen-born *S*-locus cysteine rich protein (SCR) in an allele-specific manner, then triggers a signaling cascade within the stigma epidermal cell that finally leads to the failure of self-pollen grains to hydrate and germinate [7, 25, 26]. During processes following compatible pollination, several RLKs are

the essential receptors for the female attractant AtLURE1 for micropylar pollen tube guidance [27], such as Lost in pollen tube guidance 1 (LIP1) and 2 (LIP2) [28], Male discoverer 1 (MDIS1) and MDIS2 [29], Pollen-specific receptor-like kinase 6 (PRK6), PRK1, PRK3 and PRK8 [30]. Besides, FERONIA (FER) [31], ANXUR1 (ANX1) and ANX2 [32], Buddha's Paper Seal 1 (BUPS1) and BUPS2 [33, 34], are all evolved in regulation of normal pollen tube polar growth, proper pollen tube rupture and sperm cell discharge.

Arabidopsis thaliana genome contains more than 620 RLK members that represents nearly 2.5% of *A. thaliana* protein coding genes [18, 19], *Oryza sativa* has nearly twice as many RLK members as *A. thaliana* does [35]. Amount to 76 *AtRLK* genes are preferentially expressed in semi-*in vivo* growth pollen tubes in *A. thaliana* [28]. Thus, whether other RLKs also function in the communications between male and female tissues are still need to be further investigated. Now, the availability of genome sequences for some crucifer species are very helpful for genome-wide characterization of RLK family members: *A. thaliana* [36], *Brassica napus* [37]. Furthermore, genetic basis for *A. thaliana* transition from outcrossing to selfing had been revealed, it can be reverted to full SI by transformation with *SRK-SCR* from its relative *A. lyrata* and *A. halleri* [38-42]. Using transgenic SI plants, Matsuda et al. [43] had reported the characterization of gene expression profiles of un-pollinated (UP), compatible pollinated (CP) and incompatible pollinated (IP) papilla cells in Arabidopsis. Besides, independent mutation in *SCR* caused the self-compatibility in *B. napus* line "Westar", which transgenic line by complementing the function of *SCR* showed strong SI [44, 45]. Using this ideal material, Zhang et al. [46] had explored the gene expression in *B. napus* stigmas during compatible and incompatible pollen-stigma interactions. Releases of these transcriptional data provided an excellent opportunity for a comprehensive analysis of differentially expressed genes in response to pollination in these two species. In this study, the differentially expressed *AtRLKs* and *B. napus AtRLK* orthologs after pollination were screened at the transcriptional level in *A. thaliana* and *B. napus*, the expression pattern of the differentially expressed *AtRLKs* in *A. thaliana* were analyzed, the differentially expressed *AtRLKs* during *in vitro* pollen germination and pollen tube growth were obtained, and then were compared with that after pollination. Finally, all our results showed that 89 *AtRLKs* were differentially expressed in responding to pollination in *A. thaliana* and *B. napus*, which should be mainly caused by pollen-stigma interaction.

Results

Analysis of the *B. napus* orthologs of *A. thaliana* RLKs

The RLK family has been well characterized in *A. thaliana*, its total member was up to 624 [18, 19, 35]. As the first step to analyze their *B. napus* orthologs, the Arabidopsis *RLK* genes were downloaded from TAIR according to the former report [35]. The Arabidopsis RLK family was divided into 66 subfamilies on the basis of their difference in both the kinase sequences and domain compositions [35], the number of the members varied dramatically between different subfamilies, the largest subfamily was LRR-III consisted of 47 members, whereas, 10 subfamilies only contained 1 member, respectively (Supplementary Table S1).

Then the *B. napus* orthologs of *A. thaliana* *RLKs* were retrieved from *Brassica* database, and further identified and confirmed by BLASTP and kinase domain analysis. The gene name and E-value of the *B. napus* orthologs of *A. thaliana* *RLKs* were listed in Supplementary Table S1. Their total number was 2,583, more than 4.13-fold larger than that of *AtRLKs*. However, *B. napus* orthologs of different *Arabidopsis* *RLKs* had different expansion degrees, for example, the most expanded was AT4G21380, the number of its *B. napus* orthologs was 31, whereas, the number of *B. napus* ortholog of 28 *AtRLKs* had no expansion after the *A. thaliana*-*B. napus* split (Fig. 1). Besides, 56 *AtRLKs* had no *B. napus* orthologs, especially all members of Error_region1 subfamily. Totally, most of *AtRLKs* (452/624) had 2 to 6 *B. napus* orthologs (Fig. 1, Supplementary Table S1).

Differentially expressed *RLKs* after pollination in *A. thaliana* and *B. napus*

Matsuda et al. [43] had characterized genes expressed in *Arabidopsis* stigmatic papilla cells pre- and post-pollination, including un-pollinated (UP), compatible pollinated (CP) and incompatible pollinated (IP) papilla cells at 60 min after pollination (termed UP, CP60 and IP60, respectively). They defined differentially expressed genes (DEGs) as genes with an RPKM value showing a ≥ 3 -fold change in expression at a P -value ≤ 0.05 , and obtained 779 DEGs in CP60 and IP60 data sets compared with UP [43]. Here, we obtained 20 differentially expressed *AtRLK* genes via screening of *Arabidopsis* *RLK* family members in these DEGs. Among them, 15 were up-regulated and 2 were down-regulated in CP60 vs. UP, 12 were up-regulated and 1 was down-regulated in IP60 vs. UP. Totally, 9 were commonly up-regulated and 1 was down-regulated in both CP60 vs. UP and IP60 vs. UP (Fig. 2, Supplementary Table S2).

Zhang et al. [46] had explored time-course gene expression during compatible and incompatible pollen-stigma interactions in *B. napus* stigmas, including un-pollinated (UP), compatible pollinated (CP) and incompatible pollinated (IP) stigmas at 2, 5, 10, 20, and 30 min after pollination (termed CP2, CP5, CP10, CP20, CP30; IP2, UP5, IP10, IP20 and IP30, respectively). The DEGs (\log_2 fold changes ≥ 1 and a FDR ≤ 0.01) at all stages of pollination had been analyzed [46]. In current study, we downloaded all the DEGs data sets. To following Matsuda's restrictive conditions, we had screened differentially expressed *B. napus* *AtRLK* orthologs with a ≥ 3 -fold change. Finally, we obtained 129 *B. napus* *AtRLK* orthologs from the DEGs, 57, 57, 57, 63 and 65 were respectively up-regulated in CP2, CP5, CP10, CP20 and CP 30 vs. UP, whereas only 10 and 5 were down-regulated in CP20 vs. UP and CP30 vs. UP. Besides, 56, 56, 62, 61 and 74 were respectively up-regulated in IP2, IP5, IP10, IP20 and IP30 vs. UP, 1, 2 and 48 were down-regulated in IP2, IP20 and IP30 vs. UP (Fig. 2, Supplementary Table S2). Totally, 67 *B. napus* *AtRLK* orthologs were commonly up-regulated and 8 were commonly down-regulated in IP vs. UP and CP Vs. UP (Supplementary Table S2).

129 *B. napus* orthologs corresponded to 79 *AtRLKs*. Compared with that in *A. thaliana*, 106 *B. napus* orthologs, corresponding to 59 *AtRLKs*, were only detected in *B. napus* DEGs data sets (Supplementary Table S2). 23 *B. napus* orthologs and their corresponded 10 *AtRLKs* were respectively contained in *B.*

napus and *A. thaliana* DEGs data sets, and their expression level change tendency following pollination were identical in these two species. In addition, 10 *AtRLKs* were only found in *A. thaliana* DEGs data sets (Supplementary Table S2). Totally, no matter in *A. thaliana* or in *B. napus*, the number of the up-regulated *RLKs* in both CP vs. UP and IP vs. UP were far more than that of the down-regulated *RLKs* (Fig. 2, Supplementary Table S2). Especially in CP2, CP5 and CP10 vs. UP, the number of the up-regulated *B. napus* orthologs were all 57, whereas, none was down-regulated. Only at the time point of 30 min after incompatible pollination, the down-regulated *B. napus* orthologs were amount to 48, but still less than the number of the up-regulated, 74.

Expression patterns of the differentially expressed *AtRLKs* in *A. thaliana*

In the following sections, the differentially expressed *B. napus AtRLK* orthologs were represented by their corresponded *AtRLKs* to facilitate further bioinformatics analysis. Combined with that only found in *A. thaliana* DEGs data sets, the total number of differentially expressed *AtRLKs* was 89 (Supplementary Table S2). Klepikova et al. [49] had analyzed global gene expression during development of *A. thaliana* in 79 samples covering many stages, from embryogenesis to senescence, and diverse organs. We had downloaded the expression data of the 89 *AtRLK* genes (Supplementary Table S3), and found that Klepikova et al. [49] had not detected AT4G39110 gene presented in all samples. Then we analyzed its expression via eFP Browser (Arabidopsis eFP Browse 2.0), found it was preferentially expressed in mature pollen. Subsequently, we constructed the heat map for the reminding 88 *AtRLK* genes expression in those 79 samples (Fig. 3). There were only 29 *AtRLKs* preferentially expressed in opened anthers (F.AN) and in anthers of mature flower before opening (F.AN.ad), all these genes grouped together in one branch. In addition, AT4G25390 enriched in F.AN and F.AN.ad, and AT5G46080 enriched in F.AN and petiole of the senescent leaf (L.PET.sn) (Fig. 3, Supplementary Table S3). Among the rest 57 *AtRLKs*, the most had only a very low expression level in F.AN and F.AN.ad, especially that some of them were almost not present in these two samples, such as AT1G21230, AT3G24790, AT4G23130 and AT5G60900 (Fig. 3, Supplementary Table S3). Besides, AT2G39660, AT5G61560 and AT2G41820 only highly enriched in stigmatic tissue (STI) (Fig. 3, Supplementary Table S2, S3). Excluding that only found in *A. thaliana* DEGs data sets, *B. napus* orthologs of 79 *AtRLKs* showed obvious fold changes of their expression level at different time points after pollination (Supplementary Table S2).

Pina et al. [47] had analyzed the transcriptome of Arabidopsis hydrated pollen, leaves, seedlings and siliques. Here, we had download the gene expression data, and defined gene as hydrated pollen-enriched gene if its expression level was at least 2-fold higher than that in the reminding three tissues. Finally, we totally got 1,234 hydrated pollen-enriched genes (Supplementary Table S4). Among them, only 29 *AtRLKs* also co-existed in the differentially expressed *AtRLKs* after pollination (Fig. 4). Compared with the former 29 *AtRLKs* preferentially expressed in F.AN and F.AN.ad (Fig. 3), 28 were identical, and the different were AT4G39110 and AT3G26940. AT4G39110 significantly enriched in hydrated pollen in comparison to leaves, seedlings and siliques in the Pina et al. [47] study, was not detected in all 79 samples in Klepikova

et al. [49] analysis. Conversely, AT3G26940 preferentially expressed in F.AN and F.AN.ad in Klepikova et al. [49] analysis, was not present in the 4 tissues used in the Pina et al. [47] study. Among all these 30 F.AN and F.AN.ad preferentially expressed or hydrated pollen-enriched *AtRLKs*, 4 were only up-regulated in CP60 or IP60 vs. UP in *A. thaliana* (Supplementary Table S2). The expression level of the rest 26 *AtRLKs* (or their *B. napus* orthologs) obviously varied at different time points after pollination, even if comparing with that at the time point of 2 min post-pollination, 1 was down-regulated and 25 were up-regulated (Supplementary Table S2).

Comparison of the differentially expressed AtRLKs after pollination with that during in vitro pollen germination and pollen tube growth

Wang et al. [48] had analyzed the transcriptome of *A. thaliana* mature pollen (MP), *in vitro* hydrated grains (HP) and pollen tubes grown (PT), they designed transitions from MP to HP and from HP to PT as PG and PTG, and finally obtained 326 DEGs (fold changes > 1.63, *P*-value < 0.01) during PG, 1,490 DEGs during PTG, respectively. In this study, we had screened *AtRLKs* with a ≥ 3 -fold change from DEGs, and obtained only 1 differentially expressed *AtRLK* during PG and 19 during PTG (Supplementary Table S5). Their number were far less than that of the differentially expressed *AtRLKs* after pollination.

Among all the differentially expressed *AtRLKs* after pollination and during *in vitro* pollen germination and pollen tube growth, no *AtRLK* showed obvious expression level changes both during PG and after pollination. Only 7 *AtRLKs* were shared by DEGs during PTG and after pollination, accounting for about 7.87% and 36.84% of the differentially expressed *AtRLKs* after pollination and during PTG, respectively (Fig. 5). Of these 7 *AtRLKs*, both AT4G25390 and AT3G24790 were up-regulated during PTG and after compatible pollination. AT4G13190 was up-regulated during PTG and after incompatible pollination. AT2G33580 and its *B. napus* ortholog were up-regulated during PTG and after compatible and incompatible pollination (Supplementary Table S2, S5). AT3G23750 was up-regulated during PTG, but its *B. napus* ortholog was down-regulated after incompatible pollination. AT1G16760 and AT3G20530 were down-regulated during PTG, but their *B. napus* orthologs were up-regulated after compatible and incompatible pollination (Supplementary Table S2, S5).

Discussion

The dry stigma of Brassicaceae species could accept compatible pollen while reject self-incompatible pollen from the same species or pollen from unrelated species, the polarized secretion in the dry stigmatic papillae cells after pollination is a highly regulated process [5, 7-9]. Unlike sperm cells in animals, the sperms in flowering plants are immobile, they must be exactly delivered to the ovule-enclosed female gametophyte in the ovary by guided pollen tube growth for successful double fertilization [16, 17]. All these mentioned processes require extensive communications between male and female organs and tissues. Although several RLKs had been proved to play key roles in these communications [16, 17, 22,

23], a family-wide analysis on *RLK* genes expression level changes after pollination reminded to be performed. To improve this situation, here we finished a comprehensive analysis on differentially expressed *RLKs* in responding to pollination in *A. thaliana* and *B. napus*, aiming to provide useful information for further analysis on *RLKs*' function in male-female communication.

Differentially expansion of *B. napus* *AtRLK* orthologs

The *B. napus* genome contained about 101,040 genes, 3.96 times larger than that of *A. thaliana* [36, 37]. Here, we found the number of *B. napus AtRLK* orthologs was over 4.13-fold larger than that of Arabidopsis *RLKs*. This should be not a simple consequence of the predicted gene number in *B. napus* genome larger than that in *A. thaliana*, for the reason that the number of *B. napus* orthologs varied dramatically between different Arabidopsis *RLKs* (Fig. 1, Supplementary Table S1). The divergence of Arabidopsis and Brassica had occurred about 14.5 to 20.4 million years ago [50], *B. napus* was formed about 7,500 years ago by hybridization between *B. rapa* and *B. oleracea*, followed by chromosome doubling [37]. It had been proved that both tandem and large-scale duplications mainly contributed to the expansion of the *RLK* family within Arabidopsis, while tandem duplications played a major role in the *RLK* expansion in rice [19, 35]. Thus, although we had not analyzed the mechanism for *RLK* family expansion in *B. napus*, we thought *RLK* family should undergo their independent evolution and expansion after *A. thaliana-B. napus* split, which might result in the dramatic variation of *B. napus* orthologs numbers of different Arabidopsis *RLKs*.

Differentially expressed *RLKs* after pollination in *A. thaliana* and *B. napus*

Matsuda et al. [43] had analyzed the differentially expressed genes in Arabidopsis papillae cells at 60 min post pollination, but some processes had finished at this time point, including polarized secretion in stigmatic papillae, pollen adhesion, hydration, germination and penetration [51-53]. Fortunately, Zhang et al. [46] had explored gene expression in *B. napus* stigmas at multiple time points after pollination, which was very helpful for the analysis of the consecutive changes of gene expression during the early stages of pollen-stigma interaction. Here, we made a comprehensive analysis on the differentially expressed *AtRLKs* and *B. napus AtRLK* orthologs after pollination. We found that some differentially expressed *AtRLKs* were only detected in *A. thaliana*, some only in *B. napus*, which maybe be caused by the different time points used in these two studies. However, when *AtRLKs* and its *B. napus* orthologs were detected in *A. thaliana* and *B. napus* DEGs data sets, their change tendencies were identical (Fig. 2, Supplementary Table S2). We also found that the number of up-regulated *RLKs* after pollination was far larger than that of the down-regulated, no matter after compatible or incompatible pollination in both *A. thaliana* and *B. napus*, which meant some biological processes should be activated by pollination, as we known, the SI signaling cascade, polarized secretion and pollen hydration, and so on (Fig 2, Supplementary Table S2).

After compatible pollination, a violent change of *RLKs* occurred at 2 min, and then the number of the varied *RLKs* had no change at 2, 5 and 10 min, subsequently, a moderate increase was detected at 20 and 30 min, a drastic reduction was finally detected at 60 min. This change tendency of the number of varied *RLKs* was in accordance with the morphologic observations. Secretory activity in stigmatic papillae of *A. thaliana* and *B. napus* was rapidly induced by compatible pollen [53]. In addition, both *A. thaliana* and *B. napus* compatible pollen had hydrated at 4 min post pollination [51, 52]. Therefore, multiple processes including pollen adhesion, foot formation, polarized secretion and hydration should have occurred within 4 min, the violent changes of *RLKs* at 2 min should mainly be involved in those processes. Hydration would continue to 10 min following pollination [51], it might be the main reason for no change of the varied *RLKs* number at 2, 5 and 10 min. Following, the pollen tube had emerged at 20 min post pollination, and the pollen tube penetrated the papillae cell wall within 30 min after pollination [53-55], the interaction between stigma and the emerged pollen tube might result in the moderate increase of the number of the varied *RLKs* during corresponding stages. During 30 min to 60 min, the former processes had completed and pollen tube continued to grow, which may be responsible for the drastic reduction of the number of the varied *RLKs* at 60 min. The change tendency of the number of varied *RLKs* after incompatible pollination was very similar with that after compatible pollination. A minor difference was that a moderate increase in the varied *RLKs* number emerged from 10 min after incompatible pollination. Although we had known a signaling cascade within stigmatic papillae was induced by incompatible pollen [7, 26], the polarized secretion in the dry stigmatic papillae cannot be induced by incompatible pollen, and the pollen hydration and germination were inhibited [51, 53]. In our knowledge, morphologic observations need to be further performed to illustrate the changes the number of the varied *RLKs* at different time points after incompatible pollination.

Differentially expressed *RLKs* after pollination should be mainly induced by pollen-stigma interaction

The major differentially expressed *RLKs* after pollination were up-regulated, to explore the reasons, their expression pattern and the changes of their expression level during *in vitro* PG and PTG were analyzed. Based on Klepikova et al. [49] and Pina et al. [47] studies, 29 of 89 differentially expressed *AtRLKs* preferentially expressed in F.AN and F.AN.ad (Fig. 3, Supplementary Table S3), also only 29 of the 89 were hydrated pollen-enriched (Fig. 4, Supplementary Table S4), there was only 1 *RLK* different with the former 29 *AtRLKs*. Including these F.AN and F.AN.ad preferentially expressed or hydrated pollen-enriched *RLKs*, amount to 79 *AtRLKs* corresponding to 129 *B. napus* orthologs showed obvious expression level changes at different time points after pollination, even if comparing with that at the time point of 2 min post-pollination in *B. napus* (Supplementary Table S2). Furthermore, excluding these F.AN and F.AN.ad preferentially expressed or hydrated pollen-enriched *RLKs*, the most of the reminding differentially expressed *RLKs* after pollination had a very low expression level in F.AN and F.AN.ad, some of them were even not present in these two samples (Fig. 3, Supplementary Table S3). Thus, all these results indicated

that the up-regulated *RLKs* after pollination should not be primarily caused by addition of pollen-enriched *RLKs*.

Based on Wang et al. [48] study, only 7 *AtRLKs* were shared by differentially expressed *AtRLKs* after pollination and during pollen tube growth, accounting for about 7.87% of that after pollination. In addition, among these 7 *AtRLKs* AT4G13190 and AT2G33580 were up-regulated during PTG, but their expression levels were up-regulated after incompatible pollination. As we all known, the germination of the incompatible pollen was inhibited on the dry stigma surface. Furthermore, both AT1G16760 and AT3G20530 were down-regulated during PTG, but their *B. napus* ortholog were up-regulated during the stages of compatible and incompatible pollination (Supplementary Table S2, S5). Therefore, the low repetition of differentially expressed *AtRLKs* after pollination with that during *in vitro* pollen germination and pollen tube growth, and the difference on the expression level change tendency of the shared *AtRLKs* meant that the up-regulated *AtRLKs* after pollination should not be mainly contributed by pollen germination and pollen tube growth. Taken together, all the above results suggested that the differentially expressed *RLKs* after pollination should be mainly induced by pollen-stigma interaction.

Functions of the differentially expressed RLKs in male-female communication

Among the differentially expressed *AtRLKs* after pollination, AT5G28680 was ANX2, and AT4G39110 was BUPS1. Former report had proved that ANX2 and ANX1 were male factors controlling pollen tube behavior by directing rupture at proper timing [32, 56]. Further analysis showed that ANX1 and ANX2 interacted with BUPS1 and BUPS2 to form the ANX-BUPS receptor complex, which bound to the pollen tube-expressed RALF4/19 to maintain pollen tube integrity, once RALF4/19 was replaced by the ovule-produced RALF34, pollen tube bursting would be triggered [33, 34]. AT5G16500 and AT3G02810 were LIP1 and LIP2, respectively. They were essential components of the pollen tube receptor complex to perceive the female signal AtLURE1 for micropylar pollen tube guidance [28]. AT5g45840 and AT4G18640 were MDIS1 and MDIS2. These two proteins along with MDIS1-interacting receptor like kinase MIK1 and MIK2 also participated in micropylar pollen tube guidance, especially, MDIS1 and MIK directly bound to AtLURE1 [29]. AT5G35390, AT3G20190 and AT1G50610 were PRK1, PRK4 and PRK5. PRK1, PRK2 and PRK5 were involved in the control of polarized pollen tube growth [57], PRK1, PRK3, PRK6 and PRK8 functioned synergistically in sensing of LURE1 in Arabidopsis [30]. Of the above studied *AtRLKs*, several were excluded from the differentially expressed *AtRLKs* after pollination, such as BUPS2, PRK6. We thought the main reasons were that their expression level showed no obvious changes after pollination or their change fold was < 3. Another well characterized RLK was SRK, which also was excluded from the current study. Firstly, *A. thaliana* Columbia strain contained a non-functional *SRK* [58]. Secondly, *BnSRK* was highly expressed in un-pollinated stigma, but its expression level showed no obvious changes after pollination [46]. In our opinion, the following work efforts should focus on the functional analysis of the rest differentially expressed *AtRLKs* in male-female communications, aiming to

strengthen our understanding of the molecular and cellular events behind the pollination in the Brassicaceae species.

Conclusion

In present study, we reported that 89 *AtRLKs* showed obvious expression level changes after pollination in *A. thaliana* or in *B. napus*, which should be mainly induced by pollen-stigma interaction, not by addition of pollen-enriched *RLKs* or pollen germination and pollen tube growth.

Materials And Methods

Sequence retrieval and analysis of the Arabidopsis RLK family members and *B. napus* *AtRLK* orthologs

We downloaded the names and sequences of Arabidopsis RLK family members from TAIR (<https://www.arabidopsis.org/>) according to the Supplement A online of Shiu et al. [35] report. Following Shiu et al. [35], we divided the Arabidopsis RLK family into subfamilies on the basis of their difference in both the kinase sequences and domain composition. For the subfamily names and members, see Supplementary Table S1.

B. napus AtRLK orthologs were firstly retrieved from *Brassica* database based on genome annotation (<http://brassicadb.org/brad/searchAll.php>). Then to further confirm the obtained *B. napus* orthologs, we used *AtRLK* protein sequences as queries to conduct BLASTP searches against the predicted proteins of *B. napus* with a permissive E-value cutoff of 3E-06 (<http://brassicadb.org/brad/blastPage.php>). The newly emerged *B. napus* orthologs were evaluated for the presence of the complete kinase domain against repository of the conserved domain database (<http://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>). The repetitive genes in genome annotation and BLAST analysis results were deleted according to their E-value. For the name and E-value of *B. napus AtRLK* orthologs, see Supplementary Table S1.

Differentially expressed *AtRLKs* and *B. napus AtRLK* orthologs after pollination in *A. thaliana* and in *B. napus*

Matsuda et al. [43] had characterized the differentially expressed genes (DEGs) as genes with an RPKM value showing a ≥ 3 -fold change in expression at a P -value ≤ 0.05 in Arabidopsis stigmatic papilla cells at 60 min after compatible and incompatible pollination compared with the un-pollinated papilla cells. Here, we downloaded the DEGs data sets from the Supplementary Table S1 of Matsuda et al. [43] report. Then we obtained all the differentially expressed *AtRLK* genes via screening of Arabidopsis RLK family members in these DEGs. For the name and change tendency of the differentially expressed *AtRLKs* following pollination, see Supplementary Table S2.

Zhang et al. [46] had explored the differently expressed genes with \log_2 fold changes ≥ 1 and a FDR ≤ 0.01 in *B. napus* stigmas pollinated with compatible and incompatible pollen at multiple time points (including 2, 5, 10, 20 and 30 min after pollination) compared with the un-pollinated *B. napus* stigmas. We downloaded the DEGs data sets from the Supplemental File S2 of Zhang et al. [46] report. Following Matsuda's restrictive conditions, we screened all the differentially expressed *B. napus AtRLK* orthologs with a ≥ 3 -fold change against the DEGs data sets. For the name and expression levels of the differentially expressed *B. napus AtRLK* orthologs at different time points after pollination, see Supplementary Table S2.

Expression pattern analysis of the differentially expressed AtRLKs in *A. thaliana*

We downloaded the expression data of the differentially expressed *AtRLKs* in response to pollination in 79 Arabidopsis samples from TraVA (<http://travadb.org/browse/DeSeq/>). For gene expression levels and samples descriptions, see Supplementary Table S3. Then the gene expression data that were not obtained from TraVA were analyzed via eFP Browser (Arabidopsis eFP Browse 2.0). We constructed the heat map for *AtRLKs* in these 79 samples using online Morpheus (<https://software.broadinstitute.org/morpheus/>).

We downloaded the transcriptome of hydrated pollen, leaves, seedlings and siliques of *A. thaliana* from Supplementary Table 1 of Pina et al. [47] report. Here we defined gene as hydrated pollen-enriched gene if its expression level was at least 2-fold higher than that in leaves, seedlings and siliques. For the details pertaining to hydrated pollen-enriched genes, see Supplementary Table S4. The co-existed *AtRLKs* in hydrated pollen-enriched genes and the differentially expressed *AtRLKs* in response to pollination were analyzed using online Venny 2.1.0 (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>).

Differentially expressed AtRLKs during in vitro pollen germination and pollen tube growth

We downloaded all the differentially expressed genes with fold changes > 1.63 (P -value < 0.01) during *in vitro* Arabidopsis pollen germination (PG) and pollen tube growth (PTG) from the Supplemental Table S2 of Wang et al. [48] report. Following Matsuda's restrictive conditions, in this study we screened *AtRLK* genes with a ≥ 3 -fold change via screening of Arabidopsis RLK family members in these DEGs data sets using online Venny 2.1.0 (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>). For the details pertaining to the differentially expressed *AtRLKs* during PG and PTG, see Supplementary Table S5. Then they were compared with the differentially expressed *AtRLKs* in response to pollination.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qiguo Gao conceived the study, Xingyu Chen and Yuming Dong analyzed the *B. napus* orthologs of *A. thaliana* *RLKs*, Yupeng Jiang analyzed the differentially expressed *RLKs* after pollination, Chen Kuang and Xiaoli Wang help to perform the expression pattern analysis, Qiguo Gao made the comparison of the differentially expressed *AtRLKs*, Qiguo Gao, Yupeng Jiang and Xingyu Chen prepared the manuscript. All authors read and approved the final manuscript for publication.

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Additional File Legend

Additional file 1: Table S1 Statistics of the *B. napus* orthologs of *A. thaliana* RLKs

Additional file 2: Table S2 The differentially expressed *RLK* genes in response to pollination in *A. thaliana* and *B. napus*

Additional file 3: Table S3 Expression of 89 *AtRLKs* in 79 diverse samples of *A. thaliana*

Additional file 4: Table S4 Hydrated pollen-enriched genes

Additional file 5: Table S5 Differentially expressed *AtRLKs* during PG and PTG

Figures

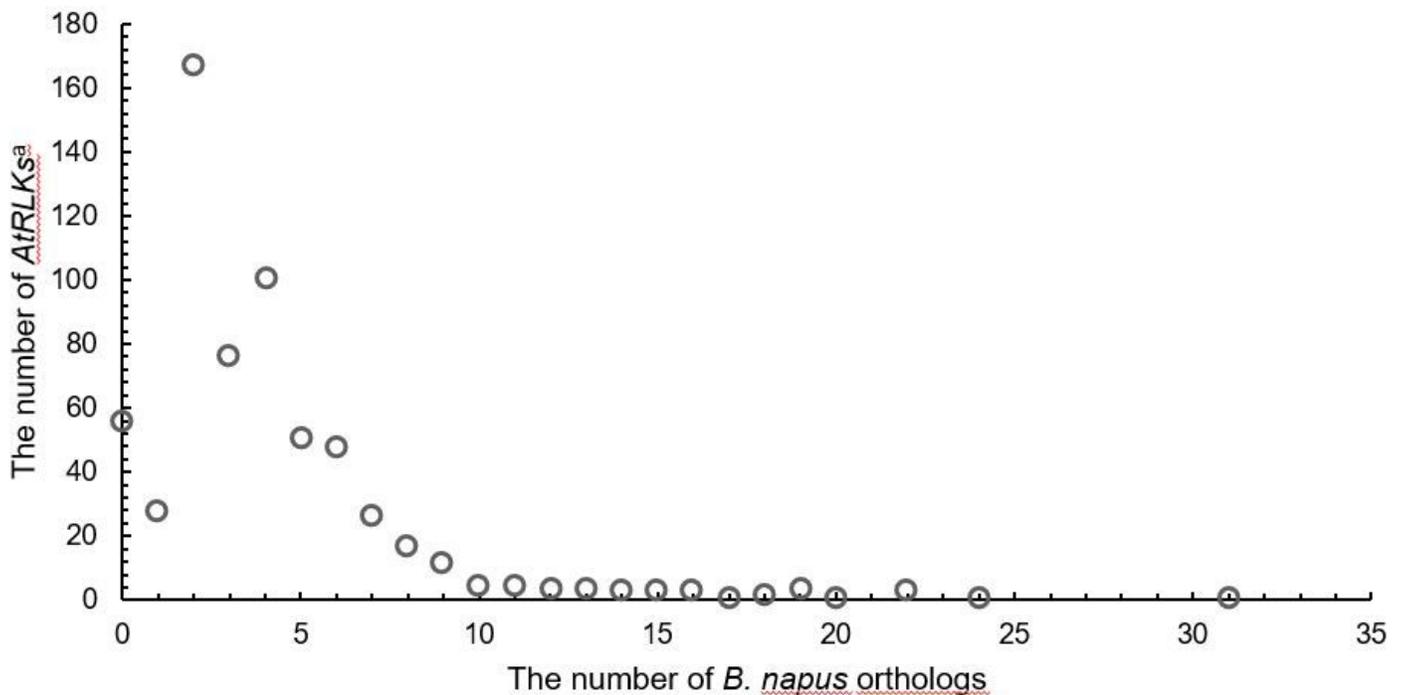


Figure 1

Statistics of the number of *B. napus* orthologs of *A. thaliana* RLK

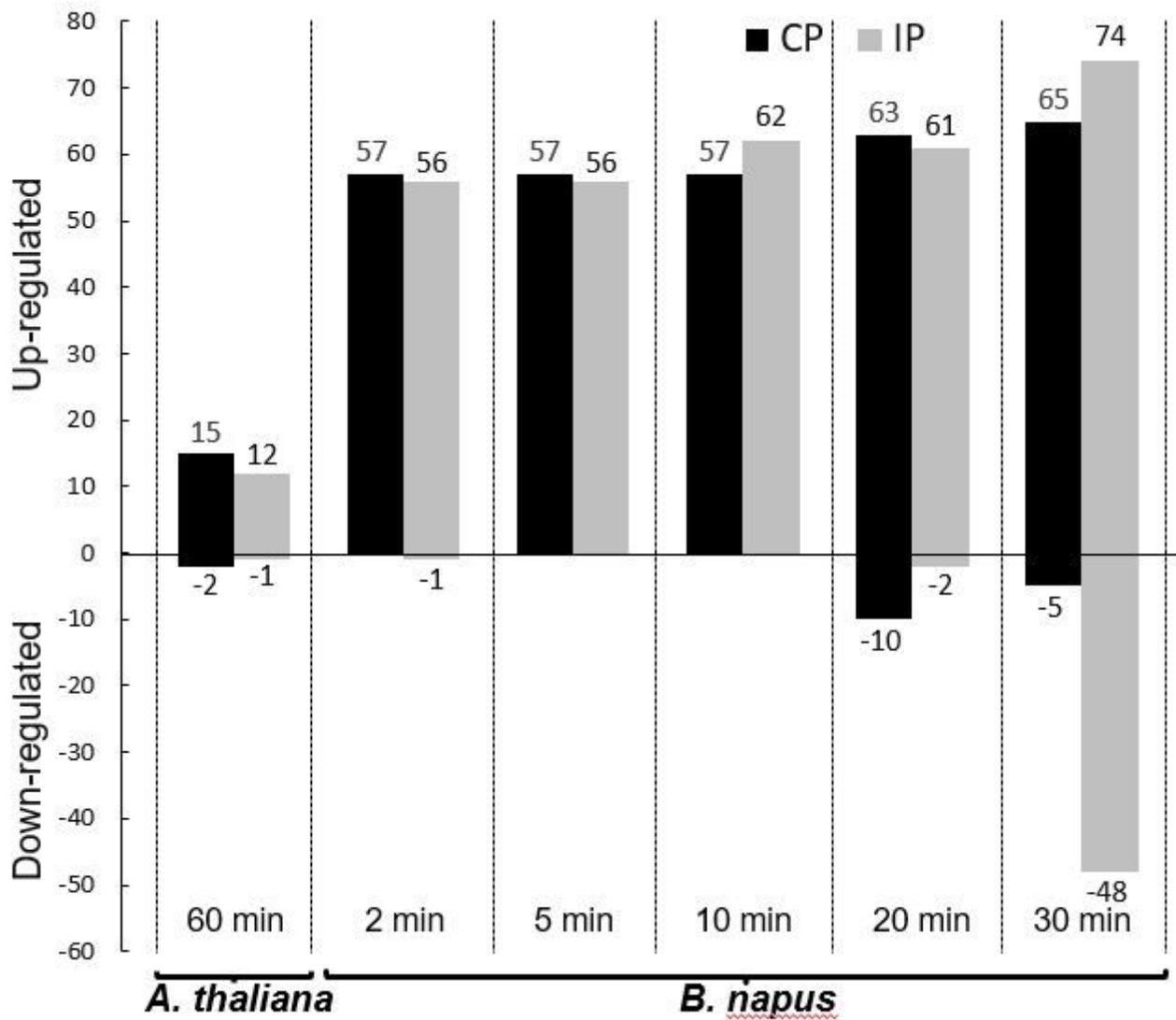


Figure 2

The differentially expressed AtRLKs or *B. napus* orthologs identified in CP and IP samples

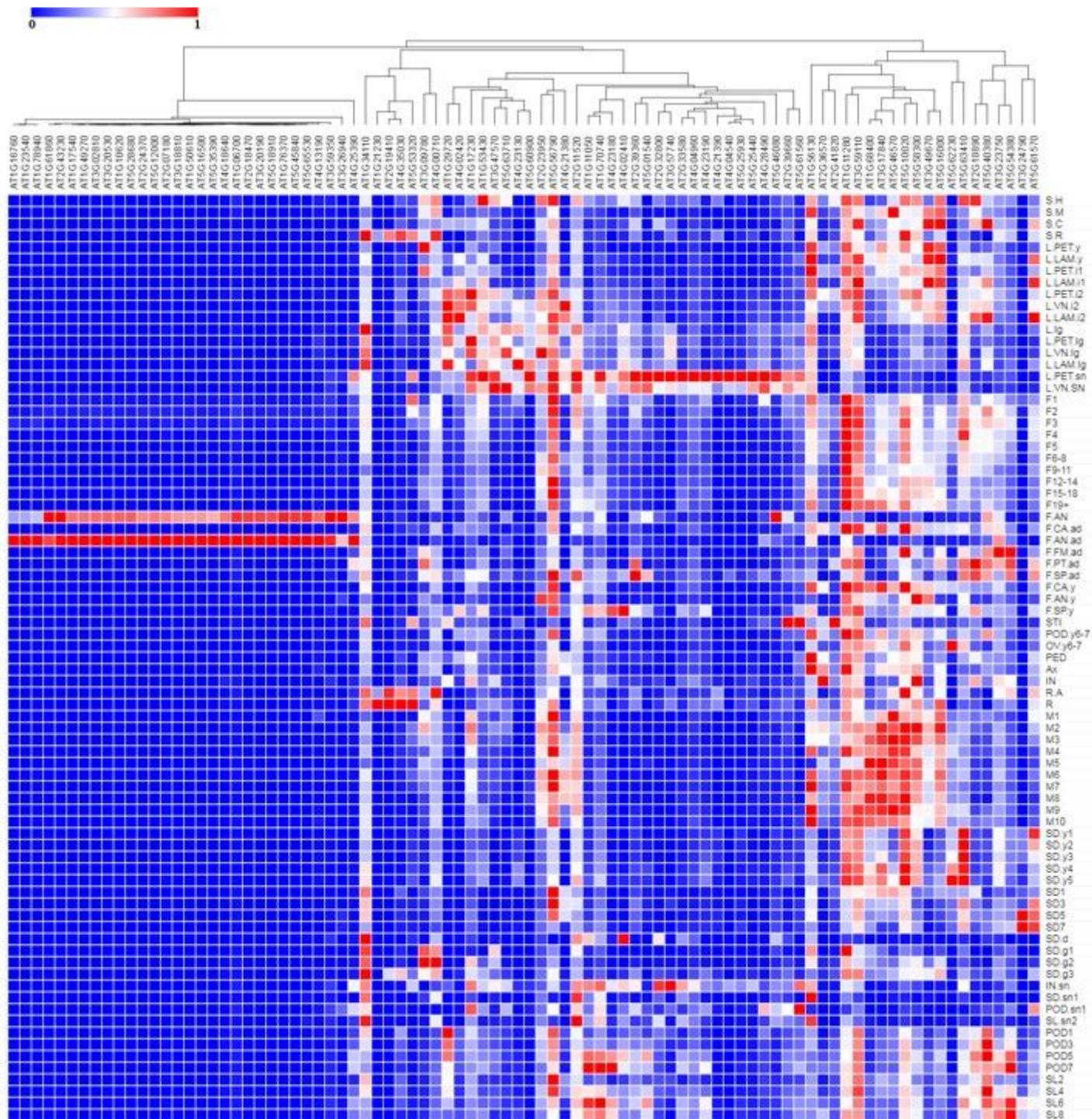


Figure 3

Heat map representation of 88 AtRLKs in 79 diverse samples of *A. thaliana*

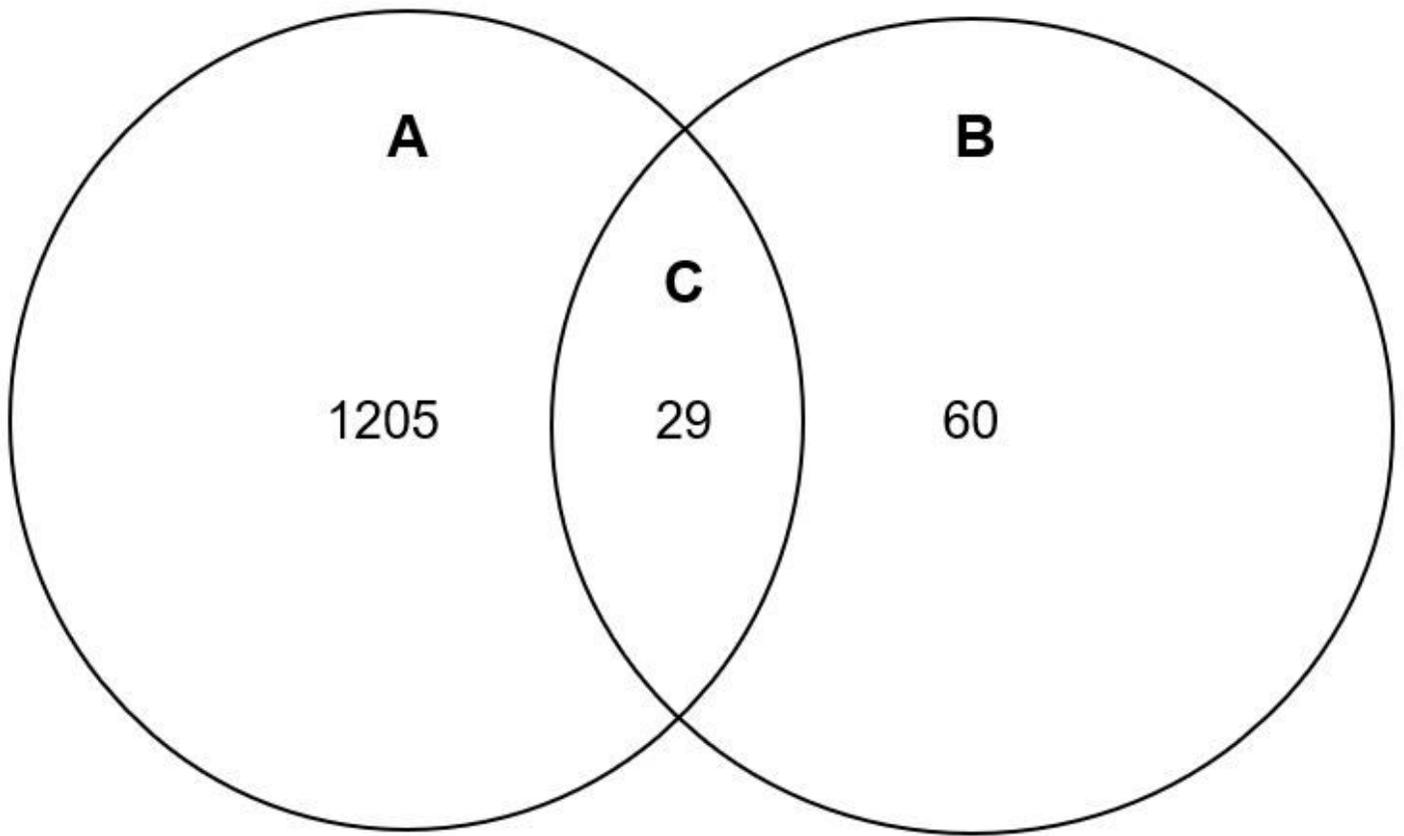


Figure 4

Comparison of hydrated pollen-enriched genes with the differentially expressed AtRLKs after pollination

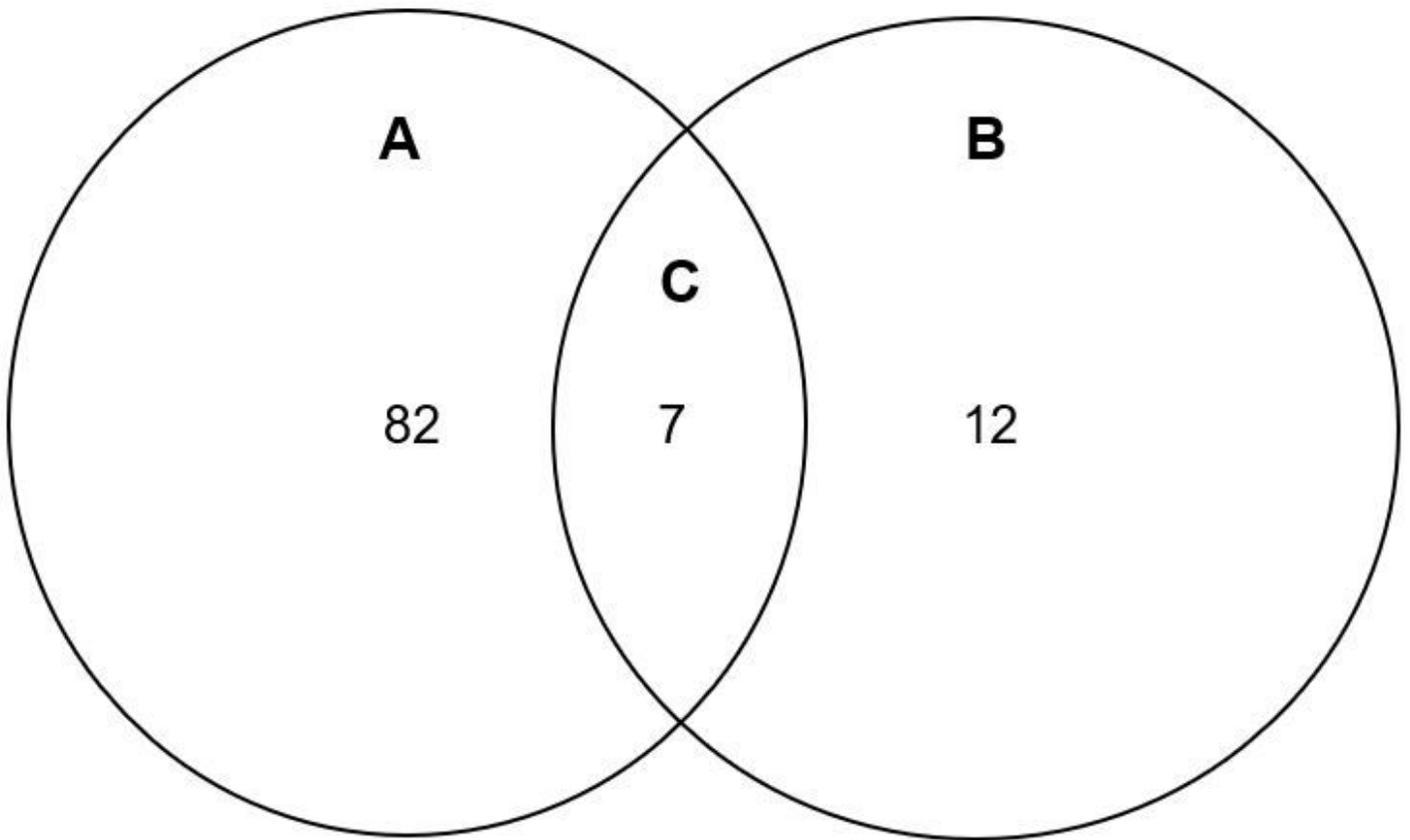


Figure 5

Comparison of differentially expressed AtRLKs during PTG with that after pollination

Supplementary Files

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